Agassiz (A.) & Whitman (C. O.)

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NEWPORT MARINE LABORATORY.

XIV.—ON THE DEVELOPMENT OF SOME PELAGIC FISH EGGS.

PRELIMINARY NOTICE.

By ALEXANDER AGASSIZ and C. O. WHITMAN.

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IV.

CONTRIBUTIONS FROM THE NEWPORT MARINE LABORATORY, COMMUNICATED BY ALEXANDER AGASSIZ.

XIV. ON THE DEVELOPMENT OF SOME PELAGIC FISH EGGS. — PRELIMINARY NOTICE.

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Read May 14th, 1884.

Considerable attention has in late years been paid to fish eggs found floating on the surface, more especially since the establishment of various Fish Commissions to study the history of the sea-fishes. As early as 1868, Malm[1] raised the eggs of a species of Flounder by artificial fecundation, and found them to float on the surface at first, and in the later stages of development to sink gradually below the surface. Sars,[2] in 1869, found that the eggs of the Cod floated on the surface. Haeckel,[3] during a visit to Corsica, in 1874, also found pelagic fish eggs which he referred to some Gadoid: he subsequently found the same eggs at Nice, in 1876. E. van Beneden,[4] in 1874, studied pelagic fish eggs at Villa Franca. Kupffer,[5] in 1868, published some interesting investigations on pelagic fish eggs found in the harbor of Kiel.

Mr. Ryder[6] and Colonel McDonald of the United States Fish Commission observed that the eggs of the Spanish Mackerel were found floating on the surface.

In 1879 and in 1882 A. Agassiz[7] published some preliminary results on the pelagic fish eggs he had raised during the past twenty

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[1] Malm, A. W. Svenska Vetensk. Akad. Handl., VII., 1867 and 1868.

[2] Sars, G. O. Indberetninger til Departementel for det Indre. Christiania, 1869.

[3] Haeckel, E. Die Gastrula und die Eifurchung. Jena Zeitschr., IX., 1875.

[4] Van Beneden, E. Quart. Journ. Mic. Sci., 1878, p. 41.

[5] Kupffer, C. Archiv für Mikr. Anat., 1868, p. 209.

[6] Ryder, J. A. Bull. U. S. Fish Com., I., p. 136, 1881.

[7] Agassiz, A. Proc. Am. Acad., vol. xiv., 1878, p. 1, and vol. xvii., 1882, p. 27.

years, some of them belonging to the Flounder, to Ctenolabrus, to Cottus, to Lophius, and to Tautoga.

According to Emery,[8] the eggs of Fierasfer are also pelagic. Kingsley and Conn[9] have also studied the pelagic eggs of Ctenolabrus; they state that Mr. Van Vleck has observed those of Merlucius, and also figured an egg with an oil globule; and finally Hensen[10] has published a most interesting paper on the occurrence of the eggs of a few of the fishes of the Baltic at the surface.

During the season of 1883 a good deal of the difficulty, and consequent confusion, existing in distinguishing the many species of pelagic eggs met with during the summer at Newport, has been evercome, and we are now able to distinguish no less than twenty-two species of pelagic eggs, nearly all of which have been referred to some of the many young stages of osseous fishes which have been collected at the surface for a series of years.

The differences between these pelagic eggs are very slight, and the greatest possible care is necessary not to confuse eggs of very dissimilar fishes. I may give as an instance that the eggs of *Ps. melanogaster* and of an undetermined Flounder had, till this year, been confounded with those of Ctenolabrus; those of the Brown Flounder with those of two species of osseous fishes as yet undetermined; those of a species of undetermined Flounder with those of Hemitripterus; and those of the Sienna Flounder with those of the Yellow Flounder. As these different pelagic eggs must go through extensive changes in the course of their development, changes in the appearance and growth of pigment spots of the body and of the yolk, it is almost impracticable, without any extensive series of sketches, to establish with certainty the identity or difference of closely allied eggs. Hensen has called attention to the ellipsoidal shape of some of the pelagic fish eggs; this is particularly striking in the egg of an Osmerus (?); in this the difference between the longer and the shorter axis can be detected by the eye. The yolk mass of this egg is remarkable for being segmented in large polygonal cells; a similar, but incomplete segmentation occurs in the eggs of the Brown Flounder.

The pigment spots of the surface of the yolk, and those characteristic of different species of fish embryos, begin to make their appear-

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[8] Emery, C. Fierasfer. Arbeit aus d. Zool. Station, zu Neapel.

[9] Kingsley, J. S., and H. W. Conn. Mem. Boston Soc. Nat. Hist., III., No. VI., 1883.

[10] Hensen, V. Bericht der Com. zur Wiss. Untersuchung der deutschen Meere, IV. Kiel, 1883.

ance at very different times in the many species we have examined. Hence, until the characteristic pigment pattern of an embryo is pretty well known, it is easy to confound the eggs of very different species. The position and shape of the otoliths and the degree of development of the pectorals become also excellent guides to the identification of eggs well advanced in their development. The differences in the young embryos on hatching are very considerable, and in these earlier stages the degree of development of the head, the proportional size of the yolk-bag, the shape of the embryonic fin, the position of the vent, and the pattern of the pigment spots, are all of great use in the identification of the species.

It is remarkable that no monstrosities have ever been picked up among the large number of pelagic eggs examined during the past twenty years, while among the eggs raised by artificial fecundation, the number of eggs which do not develop is very considerable. It is true, that unfertilized eggs, after a day or two, probably fall to the bottom, and are rapidly decomposed, or eaten by other animals. As the majority of the species of Flounders, of which the pelagic eggs have been found, live together in considerable numbers, it is probable that at the time of fecundation but few eggs escape being fertilized: the same is the case with Ctenolabrus, Tautoga, and other shallow-water species. The pelagic eggs are, of course, at the mercy of the winds and waves, and are found in the greatest abundance in the streaks formed by tidal eddies and by winds, which are everywhere on the sea-coast such excellent collecting ground for embryos of invertebrates and for other pelagic animals.

We have collected at Newport the pelagic eggs of six species of Flounders, two species of Cottus, those of Ctenolabrus, Tautoga, Osmerus, and Lophius, and have in collection the eggs of ten species of fishes as yet not determined; but they are probably the eggs of Motella, of Labrax, of Poronotus, and of the Bluefish. The exact identification of these eggs must be deferred to another season. Several of the eggs have been referred to the species of Flounder and other young fishes which were figured in former papers of Mr. Agassiz in the Proceedings of the American Academy of Arts and Sciences.

The presence or absence of an oil globule is an excellent guide in the identification of the egg; the size of this globule is, however, quite variable. In one of the species of Cottus there are many globules present, and the number of these varies from sixteen to thirty-two for this species. In another species, Hemitripterus, in which there is generally only one globule, it is not an uncommon occurrence to find two globules.

Closely allied species of Flounders are found to have eggs either with or without an oil globule. The question naturally arises how far in one and the same egg the number of globules may vary. I have followed an egg in which in some stages the number of globules varied in number from day to day. These pelagic eggs all appear to have a great number of minute fatty globules scattered through the yolk mass; these may or may not unite in a single or in many globules, or may always remain scattered in the yolk. It is undoubtedly to the presence of these minute fatty globules and the larger oil globules that the pelagic eggs owe their capacity for floating. Many pelagic eggs undoubtedly sink in the latest stages of growth.

The number of these pelagic eggs is very great. Hardly a day passes when the fishing with the surface-net does not bring in a number of eggs. The spawning season of many of the fishes which lay pelagic eggs is not very long: at any rate, the different eggs succeed one another quite rapidly, and of the twenty-one species of pelagic eggs thus far observed at Newport, none extend over a greater period than six weeks. The statement I had made, that the eggs of Ctenolabrus were found during the whole summer, rests on the incorrectness of the identification of pelagic eggs closely resembling those of Ctenolabrus, and which are collected in the last part of July and during August.

As the data for the exact determination of some of these eggs are still incomplete, we defer publishing them until they can be supplemented with the observations of another season.

During the summer of 1883, our attention was directed mainly to the earlier stages of development, embraced between the fecundation of the egg and the complete formation of the embryo. The numerous researches on the embryology of the teleostean fishes leave many points of fundamental importance yet to be settled. In evidence of this, we need only refer to the parablast theories that have appeared since the investigations of His; the contradictory views concerning the origin of the so-called "free nuclei" which appear beneath the blastoderm, and the part they play in building up the embryo; the controversies relating to the manner in which the embryo is formed; and the widely different views respecting the origin of both the mesoderm and the entoderm. Kupffer's vesicle still remains a complete mystery; and no one has thus far succeeded in giving a complete and satisfactory account of the origin of the germ-ring ("embryonic rim," Balfour).

No attempt has been made to explain *how* the alimentary canal is formed; and the precise origin of the chorda and its mode of differ-

entiation are questions which have not been exhausted. Some of the general features of the cleavage have been understood since the time of Rusconi;[11] and the researches of Ryder and Hoffmann have demonstrated the existence of polar globules, pronuclei, and karyokinetic figures, in addition to numerous other facts of both special and general importance. But it is manifest that our knowledge in this direction, invaluable as it is, is very far from having reached that degree of completeness with which we are familiar in the case of some other vertebrates, and many invertebrates. The importance of accurate and detailed study of the cleavage phenomena has been illustrated in so many cases in recent years, that it is now fast becoming unnecessary to insist upon it.

Remembering that the histogenetic sundering of the embryonic material actually begins with the cleavage, and that "jeder einzelne Entwicklungsmoment ist die nothwendige Folge des vorausgegangenen und die Bedingung des folgenden,"[12] it seems clear what course our investigations should take in order to reach satisfactory conclusions on the origin and relation of the germ-layers. But in all telolecithal vertebrate ova, especially those extreme forms in which the cleavage is restricted to a discoidal mass aggregated at one pole, the difficulties in the way of tracing the precise genealogy of individual cells soon become quite insurmountable. Notwithstanding the exceptional advantages for observation afforded by transparent pelagic fish eggs, no one has hitherto succeeded in tracing the exact genetic relationship of each cell beyond the 16-cell stage.

In passing from the 16-cell to the 32-cell stage, the central portion of the blastodisc becomes two cells deep, and on this account it becomes extremely difficult, beyond the latter stage, to trace the genesis of the individual cells in the living egg. By the aid of mounted preparations we have found it possible to obtain the complete genealogical history of each cell as far as the 64-cell stage. In leaving this stage the blastodisc becomes three cells deep in its central portion, and we have been unable to carry the complete identification of all the cells beyond this point. Fortunately, the more interesting among the concluding phenomena of the cleavage are confined to the marginal cells of the disc; and it is the history of these cells that we have been able to follow with sufficient completeness to decide one of the cardinal questions in the early development of the teleostean fishes,

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[11] Müller's Arch., 1836, p. 278.

[12] Leuckart and Bergmann. Vergl. Anat. u. Phys. d. Thierreiches, p. 19.

namely, the precise origin of what His and others have called the "parablast." The results of our investigation on this point enable us to say that this layer, the origin and nature of which have been the subject of so much controversy since the time of Lereboullet, corresponds to what is found in all vertebrate ova with discoidal cleavage, and lend strong support to the opinion that its mode of origin is in all cases essentially the same. As these results appear to be irreconcilable with those recently published by Hoffmann,[13] it is proper that we should here state the methods by which they have been reached.

Methods.

1. The successive phases of the cleavage, beginning with the moment of fecundation, were first of all followed many times over in the living egg. Profile views and optical sections were obtained by tilting the microscope, the tube being inclined at different angles between the vertical and horizontal positions, as recommended by Kingsley and Conn. The eggs were confined in a live-box, and the light controlled by the aid of Zeiss's illuminating apparatus (after Abbe). Two complete series of vertical optical sections were obtained by the camera lucida, one parallel with the longer, the other with the shorter axis of the blastodisc.

2. Mounted preparations of the blastodisc, in every stage of development from the time when the pronuclei appear up to the time when the germ-ring begins to form, were made in large numbers. I have experimented with all the hardening reagents in common use, and have failed to find any completely satisfactory method of preserving the vitellus. Even the germinal disc cannot be well preserved by any of the ordinary hardening fluids. Kleinenberg's picro-sulphuric acid, for instance, causes the cleavage products to swell, and in many cases to become completely disorganized. The embryonic stages can be hardened in chromic acid (one per cent), but the yolk contracts considerably without becoming well hardened. The best preparations of the cleavage stages have been obtained with osmic acid followed by a modified form of Merkel's[14] fluid. This fluid, as used by Dr. Eisig, consists of chromic acid (one fourth per cent) and platinum chloride (one fourth per cent) mixed in equal parts. Thus prepared it causes maceration of the embryonic portion of the egg. By using a stronger chromic acid (one per cent) and combining it as

[13] Hoffmann, C. K. Zur Ontogenie der Knochenfische, Amsterdam, 1881.

[14] Merkel. Ueber die *Macula lutea* des Menschen, Leipzig, 1870, p. 19.

before with the same volume of platinum chloride (one fourth per cent), everything may be well preserved and hardened except the yolk. But this fluid cannot be used with success unless the egg has been first killed by another agent; for eggs placed in this fluid continue to live for a considerable time, and may even pass through one or two stages of cleavage. It is therefore necessary to use some reagent that kills instantly. For this purpose a weak solution of osmic acid may be used.

The eggs are placed in a watch-glass with a few drops of sea-water, and then a quantity of osmic acid (one half per cent) equal to that of the sea-water is added. After five to ten minutes the eggs are transferred to the mixture of chromic acid and platinum chloride, and left for twenty-four hours or more. This fluid not only arrests the process of blackening, but actually bleaches the egg to a considerable extent. After this treatment it is an easy matter to separate the blastoderm from the yolk by needles; and the preparations thus obtained may be stained at once, and then treated with alcohol and mounted in balsam.

The value of such preparations must be measured by the accuracy and clearness with which they present the conditions in the living state. A careful study of the preparations shows that the method can be relied on in every particular. The osmic acid fixes the living conditions more perfectly than any other reagent at present known, and the chrom-platinum mixture completes the work of hardening without shrinkage or swelling. The finest details of the cleavage lines, the cleavage cavity, and the nuclear figures, are well preserved. The relation of the blastoderm to the protoplasmic mantle enveloping the vitellus, and all the particulars in regard to the origin of the so-called "free nuclei," are satisfactorily shown. No violence is required in order to free the blastoderm from the yolk, as a clean separation is usually effected by the action of the acids. In this separation the protoplasmic mantle invariably goes with the blastoderm, in the older as well as in the younger stages of development.

3. For sectioning, the embryonic portions of the egg need not be separated from the yolk. But before transferring the eggs from the chrom-platinum solution to the different grades of alcohol (fifty to one hundred per cent), the egg-membrane should be broken or perforated by the aid of needles on the side opposite the blastoderm, in order that the alcohol may reach the egg readily, as otherwise the membrane wrinkles badly, and often injures the embryonic portion. For the embryonic stages, the above method of hardening has not been

altogether satisfactory. The embryos are brittle, and the boundary lines between the different parts are not always sufficiently clear. A few of these stages were hardened in Perenyi's fluid,[15] and the sections have proved much more instructive than any obtained from eggs hardened in other fluids. Perenyi's fluid consists of four parts of nitric acid (ten per cent); three parts of alcohol (ninety per cent); and three parts of chromic acid (one half per cent).

Perenyi recommends leaving eggs from four to five hours in this fluid, then transferring to seventy per cent alcohol (twenty-four hours), strong alcohol (several days), and finally absolute alcohol (four to five days). Two hours' immersion is certainly sufficient for pelagic fish eggs, and probably even a shorter time would do. Among the methods of staining recommended by Perenyi may be mentioned that of mixing borax-carmine or picro-carmine with the hardening fluid. The addition of the staining fluid produces a precipitate, which should be removed by filtering. The filtered mixture both hardens and stains at the same time, which is certainly an advantage. After an immersion of a few hours in this fluid, the eggs should be transferred first to fifty per cent alcohol (five hours), then to successively higher grades. This method of hardening does not render the eggs brittle.

General Remarks on the Ovum.

Although historical summaries and general discussions must be deferred until our observations are published in full, it may be well to define at once our view in regard to what constitutes the teleostean ovum, since recent authorities differ very much on this point; and to call attention to a few general considerations which bear more or less directly on the much disputed question of the origin of the subgerminal nuclei. Bibliographical references will be limited to such as serve to make clear the points under consideration.

In harmony with the view generally received since the publication of Gegenbaur's[16] researches on meroblastic vertebrate ova, we regard the teleostean ovum as a simple cell, agreeing in all the more fundamental features of its constitution, not only with the avian, the reptilian, and the selachian ovum, but also with the holoblastic ovum of the mammal, of the amphibian, of the cyclostome, of the ganoid, and of Amphioxus.

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[15] Perenyi. "Eine neue Erhärtungsflüssigkeit." Zool. Anz., No. 119, p. 459, 1882.

[16] Gegenbaur. Müller's Arch., 1861.

We agree, substantially, with Klein [17] in the following statement: "The fact that the parablast has, at the outset, been forming one unit with what represents the archiblast (blastoderm, auct.), and, *while increasing, has spread, i. e. grown over, the yolk* which underlies the segmentation cavity, is, I think, the most absolute proof that the yolk is as much different from the parablast as it is from the archiblast." But we do not find in this a valid argument for the opinion that the yolk of the teleostean ovum has no homologue in holoblastic vertebrate ova.[18 & 19] On the contrary, we maintain, with Balfour,[20] Waldeyer,[21] and others, that the genesis of the ovum and the absence of any sharp delimitation between the protoplasm and the deutoplasm before impregnation, show conclusively that the latter is an integrant portion of the ovum. The discovery by Balfour (No. 20, pp. 57, 90) and Schultz [22] of a protoplasmic network ("Keimfortsätze," Waldeyer) extending throughout the yolk in the elasmobranch ovum, and the discovery by Van Bambeke [23] of "fines traînées protoplasmiques qui partent en rayonnant de la base du disque et plongent dans la sphère vitelline," in the unfecundated ovum of osseous fish, make it sufficiently evident that the food-yolk cannot be regarded as adventitious material in either case. When we reflect that among telolecithal vertebrate ova a complete series of graduations in the segregation of formative from nutritive material are found between the amphibian ovum and the teleostean ovum, we find it impossible to accept any theory of the constitution of the ovum which is not broad enough to include *both* extremes. The positive evidence in favor of the view here maintained does not lie, as supposed by Balfour, in the conversion of the periblast ("parablast," auct.) into a cellular layer, but *in the actual cleavage of the yolk in some teleostean ova, as first noted by Mr. Agassiz.*

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[17] Klein, E. "Development of Common Trout." Quart. Jour. Mic. Sci., XVI., p. 127, 1876.

[18] Ryder, John A. "Development of Silver Gar." Bull. U. S. Fish Com., I., p. 295, 1881.

[19] "Observations on the Absorption of the Yolk, the Food, Feeding, and Development of Embryo Fishes." Bull. U. S. Fish Com., II., p. 199, 1882.

[20] Balfour. Development of Elasmobranch Fishes, pp. 57, 89–90, 1878.

[21] Waldeyer. "Archiblast und Parablast." Arch. f. Mik. Anat., XXII., Heft 1, 1883.

[22] Schultz, Alex. "Zur Entwicklungsgeschichte des Selachiereies." Arch. f. Mik. Anat., XI., 1875.

[23] Van Bambeke. "Recherches sur l'Embryologie des Poissons Osseux." Mém. Cour. et Mém. de Sav. Étrang. de l'Acad. Roy. Belgique, XL., 1875.

The teleostean ovum affords a beautiful illustration of what Lankester[24] has designated as "precocious segregation"; for here the separation of germinal material from food-yolk becomes complete, or nearly so, before the cleavage process begins; and even the blastodisc and the periblast, at first continuous, soon part company without the direct intervention of cleavage. In these respects this ovum represents a higher type than does the elasmobranch ovum; but there is another respect in which the reverse seems to be true. The fact that the entire germ-ring enters directly into the embryo certainly points to a more primitive mode of embryonic formation than is seen in the elasmobranch ovum. In this particular the elasmobranch ovum represents an intermediate condition between the teleostean and the avian ovum. How is this fact to be reconciled with the opinion that the teleostean ovum represents a later type than that of the elasmobranch? We are of the opinion that it can be best explained on the hypothesis first suggested by Balfour,(20) that the teleosts are derived from a type of fish with a much larger ovum. The presence of an enormous mass of food-yolk in the elasmobranch ovum retards the closure of the blastopore, and the general effect is the same as if the formation of the embryo had been greatly accelerated. The result is that the embryo is already formed and constricted off from the yolk before the blastopore closes. In precisely the same way we may explain the still more extreme case of the chick. Assuming that there has been a large reduction of food-yolk in the ovum of the osseous fish, we may safely conclude that the closure of the blastopore has been correspondingly hastened; and it is the comparatively early closure which renders possible the inclusion of the entire germ-ring in the embryo. Thus the apparently earlier type of development exhibited in osseous fishes may be explained as a case of reversion.

A very marked polar differentiation characterizes the mature teleostean ovum. Hatschek[25] was the first to call attention to the universality and the early appearance of polarity in the egg; and Balfour[26] has pointed out the influence of the polar concentration of the vitellus in determining the various forms of cleavage. Von Baer[27] defines very clearly the axis of the frog's ovum, and its relation to the

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[24] Lankester. Notes on Embryology and Classification, for the Use of Students. London, 1877.

[25] Hatschek. "Pedicellina." Zeitschr. f. Wiss. Zool., XXIX., p. 502, 1877.

[26] Balfour. Comparison, etc. Quart. Journ. Micr. Sci., p. 210, 1875.

[27] K. E. von Baer. "Die Metamorphose des Eies der Batrachier." Müller's Arch., p. 484, 1834.

first cleavage planes, which he designates as *meridian*, *equatorial*, and *parallel;* but the general significance of these relations has hitherto passed unnoticed. Mark[28] comes very near to this point, when he states that the axis of the ovum is probably homologous throughout the metazoa, and adds that the "maturation spindle" always lies in this axis. We would carry the generalization one step farther, and say it is highly probable that *the first cleavage-spindle invariably lies at right angles to the axis of the ovum throughout the metazoa; and that therefore the first cleavage-plane is always a meridian plane,* at least in all cases where the first cleavage-spindle is accompanied by cleavage. As the evidence now stands, we cannot affirm that this rule is universal; but it is so general that the few cases which might be urged as exceptions, can hardly weaken its importance. Among the gastropods we find a very peculiar departure from this rule, in the case of *Nassa mutabilis*[29] and *Modiolaria* (Crenella) *marmorata* Forb.[30] It is easy to see, however, that the cleavage in these gastropods forms no real exception. The equatorial division of the ovum of Nassa, described by Bobretzky as beginning just before the meridian cleavage appears, is nothing more nor less than a *constriction* which simulates a cleavage-plane. That it is not a proper cleavage is shown by its entire behavior, and by the position of the first cleavage-spindle. But what explanation can be given of this constriction, which after appearing twice vanishes without accomplishing anything? We shall undertake later to show that this phenomenon is only one of many kindred phenomena which may all be ascribed to nuclear influence; and shall content ourselves here by comparing it with the constriction that comes and goes around the blastodisc of the teleostean ovum, during the early stages of cleavage. It is the same influence which causes the germinal protoplasm to concentrate in the form of a polar disc, and to thicken up until it forms a sort of calotte. The protoplasm gathers up around the two poles of the first cleavage-spindle, as if they were two centres of attraction; and simultaneously the outward manifestations of the tendency of each half of the disc to assume a spherical condition appear in the form of a meridian

[28] E. L. Mark. "Maturation, Fecundation, and Segmentation of Limax campestris." Bull. Mus. Comp. Zoöl., VI., Part II. No. 12, p. 512, 1881.

[29] N. Bobretzky. "Studien ü. d. emb. Entw. d. Gastropoden." Arch. f. Mik. Anat., XIII. pp. 98–105, 1877.

[30] Lovén. "Bidrag till Kännedomen om Utvecklingen af Mollusca Acephala Lamellibranchiata." Kongl. Vetensk. Akad. Handl., 1848. (Translation, Arch. f. Naturgeschichte, XV. Jahrg., 1849, p. 312.)

cleavage-groove, and a more or less distinct constriction between the margin of the disc and the yolk.

In Nassa the two blastomeres succeed in attaining a completely spherical form and the equatorial constriction is carried to the point of separation. As soon, however, as the meridian cleavage is finished, one of the blastomeres coalesces with the deutoplasmic mass, and the result is two very unequal cleavage-spheres. The second cleavage-plane, which is also a meridian plane, at right angles to the first, is accompanied by the same equatorial constriction, and followed by a coalescence which completely cancels the effect of the constriction, leaving a stage of four blastomeres. Leaving these constrictions out of account, as forming no part of the proper cleavage, we may say that the ovum of Nassa follows the general law of cleavage, in beginning with two meridian grooves.

According to Brooks,[31] the early stages of cleavage in the oyster are identical with those in Nassa. The "exceptions to the normal method of segmentation" plainly demonstrate that the trefoil and the cinquefoil stages represent, in reality, the 2-cell and the 4-cell stages.

The fact, if it be a fact, that the axis of the ovum is homologous in the higher animals, implies much more than has yet been stated. It implies that certain definite and fundamental relations are predetermined in the unsegmented ovum, among which may be mentioned constant relations, first, between the poles and the germ-layers; second, between the axis of the ovum and the axis of the embryo; and third, between both these axes and the first cleavage-planes. For reasons which do not require to be stated here, there is a much higher probability that the first one or two cleavage-planes sustain uniform relations with the axis of the ovum, than that the later planes do so. We cannot here enter into a discussion of the general bearing of these several points; but one of these, viz. the relation of the first cleavage-plane to the axis of the ovum, deserves something more than a passing notice in this connection. It is somewhat surprising, in view of the facts now before us, that recent writers have gone on talking about cleavage as if it were nothing extraordinary for it to begin with an equatorial or parallel groove. It is plainly a matter of fundamental importance, especially in ova with a pronounced polar segregation of material, whether the first cleavage-plane passes through the pole from which the polar globules issue, or at right angles to the axis

[31] Brooks. "Development of the Oyster." Studies from the Biolog. Lab., I., No. 4, 1880.

of the ovum. In one case there would be a simple division of material; in the other, a histogenetic sundering of substances, which ordinarily comes as one of the concluding events of cleavage. The natural order is unquestionably two meridian cleavages followed by an equatorial or horizontal cleavage; and we have yet to see decisive evidence that this order has been completely inverted in any single instance. Polar segregation has been carried to such an extreme in some ova that it practically amounts to a complete separation of food-yolk from the formative material; and, if we mistake not, the line of separation thus established has, in a few instances, been mistaken for an equatorial cleavage. Something of this kind has happened in the case of Nassa and Crenella, and we venture to suggest that it *may* have occurred in that of Balanus and Scalpellum.[33]

In a single octavo plate Haeckel[34] has described the development of a gastropod (*Trochus?*) and a chætopod (*Fabricia*) as far as the gastrula stage. He speaks of an axis which has its poles in the "animal" and "vegetative" halves of the ovum, and which becomes characterized either before or during the process of cleavage. The cleavage of the gastropod ovum conforms to the rule before stated; while that of the chætopod, to all appearances, proceeds on a fundamentally different plan. In Fabricia the first cleavage-plane *appears* to be an equatorial plane, separating the ectodermic from the entodermic pole. As Haeckel's figures are designed to illustrate the formation of the gastrula rather than to give the details of cleavage, it would be unjust to criticise them too closely in the latter respect. Before, however, Fabricia is put down as a real exception to the general rule, one or two points must be cleared up by further investigation. It is by no means certain that the first cleavage-plane is not meridian, instead of equatorial as it appears to be. The relation of the polar globules to the plane of division would decide the question.

Haeckel's views are, however, irreconcilable with those here maintained; for he states that the first cleavage-plane *generally* divides the ovum into two unlike parts, "a smaller animal cell, the mother-cell of the exoderm, and a larger vegetative cell, the mother-cell of the entoderm"; and cites Fabricia, the rotifera, and the gephyrea as examples.

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[32] Rabl. "Entwick. d. Tellerschnecke." Morphol. Jahrb., V., pp. 573–584, 1879.

[33] Lang. "Die Dotterfurchung von Balanus." Jena Zeitschr. f. Naturw., XII., p. 671, 1878.

[34] Haeckel. No. 3, p. 425, Pl. XXIV.

In the rotifera, according to Salensky's [35] observations on *Brachionus urceolaris*, the first cleavage-plane is equatorial; and this is followed by two meridian cleavages of the smaller sphere. Had Salensky succeeded in finding polar globules and in tracing *circumstantially* the origin of the 5-cell stage, we could have no hesitation in conceding the full force of the exception. The eggs of the rotifera are by no means favorable objects for deciding the question under consideration. No satisfactory evidence of polar globules in these ova has been obtained. If these ova develop parthenogenetically, as supposed by Cohn [36] and Huxley [37]; and if, on this account, no polar globules are produced, it might be possible to explain the appearance of an equatorial cleavage prior to the meridian planes of cleavage. The archiamphiaster which gives rise to the polar globules always lies in the axis of the ovum, and its plane of division is therefore parallel to the equator of the ovum. If the division of the archiamphiaster led to the division of the ovum, instead of the formation of a polar globule, the first cleavage-plane would be equatorial or parallel.

In regard to the gephyrea, Haeckel is certainly in error, in proof of which we may refer to the investigations of Selenka [38] on Phascolosoma, and of Spengel [39] on Bonellia. A comparison of Selenka's figs. 1 and 2, Pl. XXIX., with Haeckel's figs. 93 and 94, Pl. XXIV., will show how easily a meridian cleavage, where the polar globules have not been observed, could be mistaken for an equatorial one.

The researches of Van Beneden [40] on the maturation, fecundation, and cleavage of the mammalian ovum, of which we have thus far received only a preliminary account, leave it doubtful whether the first cleavage-spindle is parallel with the axis of the ovum or at right angles to it. He calls attention to the pronounced polarity of the ovum, but expressly states that he has not yet obtained a complete history of the first cleavage-spindle.[41] The relation of the plane of first cleavage to the axis of the ovum is therefore undetermined. If

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[35] Salensky. Zeitschr. f. wiss. Zool., XXII., p. 456, 1872.

[36] Cohn. Zeitschr. f. wiss. Zool., VII., 1856.

[37] Huxley. Trans. Micr. Soc., 1853.

[38] Selenka. Zeitschr. f. wiss. Zool., XXV., p. 444, 1875.

[39] Spengel. Mitt. a. d. Station z. Neapel, I., p. 374, 1879.

[40] E. van Beneden. "La Maturation de l'Œuf, la Fécondation et les premières Phases du Développement Embryonnaire d. Mammifères." Bull. de l'Acad. Roy. des Sci. de Belgique, December, 1875. Also in Journal de Zoologie, V., 1876.

[41] Id. "Formation des Feuillets chez le Lapin." Arch. de Biol., I., pp. 140, 141, 1880.

the first cleavage divides the ectodermic from the entodermic pole, as supposed by Van Beneden, it is most probably equatorial. If further researches prove that the cleavage begins with an equatorial groove, a very important exception to the general rule will be established. If, on the other hand, it turns out that the first cleavage is meridian, it will be difficult to reconcile this fact with Van Beneden's opinion on the destination of the first two cleavage-spheres.

The nematode ovum presents some difficulties, the importance of which we would not underestimate. Goette[42] has followed the cleavage with such accuracy and detail that he feels warranted in asserting that the first cleavage divides the ovum into two parts *which show no regular differences of size, form, or color*, but which must nevertheless be regarded as unlike in character, since one gives origin to the ectoderm and the other to the entoderm. Goette gives no information in regard to nuclear transformations, and nothing definite on the polar globules further than that they are first seen at one (aboral) end of the ovum, and that they change position so as to be of no use in orientation. The long axis is regarded as the axis of the ovum, and the first cleavage as equatorial, since it cuts the axis at right angles near the middle. Unlike most observers, Goette does not overlook the fact that this is an exception to the general mode of cleavage among the vermes; and he explains it as the result of the confinement of the ovum in a stiff ellipsoidal membrane, which prevents the ovum from elongating transversely, as it would naturally do if the cleavage plane coincided with the long axis (l. c., p. 65). Goette's observations form a most valuable contribution on the promorphology of the vermian ovum. He recognizes a homologous axis, an ectodermic and an entodermic pole, and uniform relations between the axis of the ovum and that of the embryo, and between the poles and the dorsal and ventral surfaces. All these relations become evident in the development of the nematode, the moment we take account of the fact that the cleavage process, in adapting itself to the form of the egg-membrane, causes the ovum to rotate on its transverse axis through 90°.

The very interesting discovery made by Auerbach,[43] that the pronuclei perform a rotation of 90° on an axis perpendicular to the longitudinal axis of the ovum, just before the cleavage begins, confirms the opinion that there is a transposition of cleavage-planes, at least in the case of *Rhabditis nigrovenosa* and some other nematodes.

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[42] Goette. Abhandl. z. Entw'gesch. d. Tiere, Erstes Heft, p. 59. Leipzig, 1882.

[43] Auerbach. Organologische Studien, II., p. 212, 1874.

In *Tylenchus*, *Anguillula*, and *Rhabditis dolichura*, according to Bütschli,[44] the case is different; for here the first cleavage, although at right angles to the longer axis of the ellipsoid, is meridian, if the exit of the polar globule determines the position of the axis of the ovum.

From the foregoing examples it will be seen that the exceptions in the direction of the first cleavage-plane are neither so numerous nor so serious as might at first be supposed. For the most part they are cases in which the data for exact orientation have escaped detection, either because the difficulties in the way of direct and connected observation were not surmounted, or because the attention of the observer was not brought to bear on the point we are here considering.

But why, it may be asked, should there be any uniformity in the direction of the first cleavage-plane? What are the antecedent conditions or relations which determine this uniformity? It is a well-known fact that the nuclear spindles of one cell-generation tend to arrange themselves at right angles to those of the preceding generation. The primary spindle (archiamphiaster), at the moment when it divides to form one of the polar globules, almost invariably coincides with the axis of the ovum;* and hence the first cleavage-spindle usually assumes a position perpendicular to this axis, and the corresponding cleavage falls in a meridian plane. The primary or "maturation spindles" assume a radial position, evidently in obedience to what is known as the polarity of the ovum; and the arrangement of successive spindles is regulated by the polarity of the blastomeres to which they belong, and by the interaction of these polarities. The uniformity in the direction of the first cleavage plane is then only an outward manifestation of a more fundamental uniformity in the constitution of ova, and herein lies its significance.

From this point of view, it is not the rule so much as the exception which stands in need of explanation. In a spherical ovum developing under normal conditions, there are no mechanical causes interfering with the ordinary course of events, in a manner to bring about the substitution of an equatorial for a meridian cleavage. The polar concentration of the germinal material would plainly have the contrary effect; and, when carried to the extreme seen in the teleostean ovum, would present conditions that are not only favorable to the normal position of the first cleavage-spindle, but actually unfavorable to any other position.

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[44] Bütschli. Studien ü. d. ersten Entwicklungsvorgänge der Eizelle, etc., pp. 19–22, 1876.

* Cucullanus forms an interesting exception, according to Bütschli, No. 44.

While insisting on the constancy and uniformity of promorphological relations as the foundation of all later homologies, we are not unmindful of the recent important investigations of Pflüger[45] on the influence of gravitation on the division of cells.

These investigations have been carried through with all the skill of a master hand, and they have brought to light a multitude of very astonishing facts, many of which bear directly on the points above presented. Of the accuracy of the results there is no room for a shred of doubt; but the validity of the general conclusions is, in some very important particulars, much more than doubtful. For the present, we shall limit our remarks to one or two points on which our views differ most materially from those of Pflüger. The following conclusion, for example, is based on the assumption of a complete "isotropy" of the ovum, — an assumption most emphatically contradicted by the observations.

"*Das befruchtete Ei besitzt gar keine wesentliche Beziehung zu der spätern Organisation des Thieres, so wenig als eine Schneeflocke in einer wesentlichen Beziehung zur Grösse und Gestalt der Lawine steht, die unter Umständen aus ihr sich entwickelt. Dass aus dem Keime immer dasselbe entsteht, kommt daher, dass er immer unter dieselben äussern Bedingungen gebracht ist.*"

Place this *conclusion* by the side of the following *observation*: —

"Die Embryonalanlage wird aber *stets* gefunden auf derjenigen Hälfte des lotrechten primären Meridianes, welche bei schief liegender primärer Achse die *obere* ist. Abermals entscheidet die Beziehung zur Richtung der Schwerkraft. *Die einzelnen Theile einer Meridianhälfte können nun nicht als gleichwertig betrachtet werden.* Niemals sah ich die erste Entstehung der Rusconi'schen Oeffnung und des centralen Nervensystems auf der schwarzen Hemisphäre. *Sie entstehen stets vom weissen Gürtel des tertiären Aequators aus. Hier ist der Krystallisationspunkt der specialisirten Organisation. Von hier aus entsteht der Kopftheil des Nervensystems stets in der Richtung nach dem schwarzen, der Steisstheil in der nach dem weissen Pol.*"

One of the more important among these remarkable discoveries, the general significance of which seems to have been overlooked, is the transit of Rusconi's opening across the white hemisphere of the ovum. This opening, beginning as a horizontal cleft at one point in the equator, was found to shift its position gradually *backwards* until

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[45] E. Pflüger. Archiv f. d. ges. Physiol., XXXI., pp. 311–318, and XXXII., pp. 1–79, 1883. Abstr. in Biolog. Centralblatt, III., No. 19, pp. 596–601.

it reached a point on the opposite side of the equator. This extremely interesting fact reveals a fundamental agreement between the frog and the fish in regard to the mode of formation of the embryo.

By fixing the frog's ovum in abnormal positions, at the time of fecundation or soon after, Pflüger has shown conclusively that the *direction* of the first two cleavage-planes will invariably be vertical, whatever be the position of the ovum; and from this he concludes that the line of intersection of these cleavage-planes coincides, under normal conditions, with the axis of the ovum, *for no other reason than that this axis happens to represent the direction of gravitation.* In other words, gravitation determines the vertical direction of the first cleavage-plane entirely independently of any pre-established axial relations. Now such a conclusion stands in plain contradiction with our general knowledge of cleavage; and indeed is refuted, in our estimation, by Pflüger's own observations. Why is it that the first cleavage-plane is invariably meridian in the ovum of the fish, not only in those cases where the blastodisc lies at the upper or lower pole, but also in those exceptional cases noticed by Ryder and others where it lies at one side of the vitellus? If the cleavage-plane must follow the direction of gravitation in the first instance, then why not in the third? What means this invariable *order* in the succession of the early cleavage-planes? Pflüger should be the last man, after his experiments, to declare that the order is the same because the outward conditions are the same. If it is the purpose of the cleavage merely to split up the germinal material into small pieces, and a matter of utter indifference in what order this is accomplished, how does it happen that in the teleostean ovum, according to our observations, *the first cleavage-plane coincides with the median plane of the embryo?* And is it merely a remarkable coincidence that, according to the concurrent testimony of both Pflüger and Roux,[46] precisely the same relation holds in the ovum of the frog? Or shall we infer that because this relation between the first cleavage-plane and the median plane of the embryo may be reached by two modes of cleavage, "es ist ziemlich gleichgiltig in welcher Reihenfolge die vorschreitende Zerkleinerung sich vollzieht"?

If gravitation were the sole controlling and guiding agency in cleavage, its effect ought to be *instantaneous*, and it should be possible to change the direction of a cleavage-plane already in progress. The

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[46] Wilhelm Roux. Ueber d. Zeit d. Bestimmung d. Hauptrichtungen d. Froschembryo. Leipzig, 1883.

impossibility of doing this is demonstrated by Pflüger's experiments, and herein we find an insuperable objection to his theory. Again, if one cleavage-plane has no definite and constant relation to antecedent and subsequent planes, its direction being under the exclusive control of gravitation, it should be possible to turn the second cleavage-plane out of its normal relations with the first cleavage-plane, by turning the ovum *after* the first cleavage-groove appears. Experiments directed to this end also gave negative results. The same holds true of the third cleavage, and probably of all subsequent ones.

A very suggestive fact was brought out in regard to the third cleavage. If the ovum was turned upside down immediately *after* the appearance of the second cleavage, the position of the third cleavage was not affected; but if the inversion of the ovum preceded by an hour or more the beginning of the first cleavage, then the third cleavage appeared in a parallel plane in the unpigmented half of the ovum, and this pole went on segmenting more rapidly than the black pole. The *time* required to bring about such a remarkable transposition of the third cleavage-plane suggests *a corresponding internal transposition of the active protoplasmic matrix of the ovum.* If a body constituted like the ovum is restrained by artificial means from taking its normal position, a redistribution (Umlagerung) of material must immediately set in and continue until the equilibrium is restored. The active basic portion of the ovum, having a lower specific gravity than the passive nutritive elements, would eventually recover its normal position, and thus the virtual axis of the ovum would inevitably right itself in spite of the inability of the ovum to rotate bodily. From this point of view, we may still hold that there is a constant relation between the axis and the cleavage-planes, and that the first two of these are vertical because the axis is vertical, and not because this is the direction of gravitation.

Cleavage.

Our observations on the cleavage have been most complete in the case of Ctenolabrus; for here we have followed its entire course from the moment of fecundation onward; and our observations on the living ovum have been supplemented by a study of sections, and of several complete series of mounted preparations. We have followed the cleavage phenomena with somewhat less detail in *Pseudorhombus melanogaster*, *Ps. oblongus*, and Tautoga, but with sufficient care to warrant us in saying that they are in no essential respect different from those observed in the ovum of Ctenolabrus.

From the time when the pronuclei appear to the moment when the first cleavage-groove begins, is a short period of not more than thirty minutes; but it is a period of special interest in the history of the nuclei, as will presently be seen. We have watched this period through twice on artificially fertilized ova, and not less than a dozen times on ova taken directly from the sea. The Newport Laboratory is so near the place from which most of our material was derived, that we found no difficulty in obtaining ova from five to ten minutes after fecundation.

The polar disc, to which cleavage is at first restricted, has received a variety of names. It is the "Anschwellung" of Rusconi; the "Bildungsdotter" of Reichert; the "ampoule du germe," or "vitellus formateur" of Lereboullet; the "archiblast" of His; the "blastodisc" of Haeckel; the "disque germinatif," "germinal disc," "Keim," of various writers; and is the exact equivalent of the "discus proligerus" (Von Baer) of the chick. It gives rise to the "cap," "calotte blastodermique," or "blastoderm," of later stages. We shall employ for this portion of the ovum the term *blastodisc*, since it is a word now in general use, and is not burdened with any of the theories connected with the use of the name "archiblast."

The thinner portion of the protoplasmic mantle of the ovum is also known under various names. It is called "membrane vitellaire" by Vogt; "Dotterhaut," by Oellacher; "membrane interne," "feuillet muqueux," by Lereboullet; "couche intermédiaire," by Von Bambeke; "intermediate layer," by Von Beneden; "parablast," by His; "pellicle," "yolk-hypoblast," by Ryder. As the name "parablast" has been used in very different senses by His, Klein, Hoffmann, Waldeyer, and others, it seems best to employ a new term, which is at least free from confusing associations. For this portion of the ovum we propose the name *periblast*, — a name which has the advantage of not prejudging the question of the origin and destination of the part it designates.

Blastodisc and *periblast* are simply names for two portions of one and the same envelope, which invests the vitelline sphere. In all the ova we have studied the blastodisc occupies the lower pole; but for purposes of comparison it will be convenient to speak of this as the upper, or *ectodermic pole;* and of the opposite, as the lower, or *entodermic pole.* The vertical line joining these two poles is the *axis of the ovum.*

The peculiarities in the development of the ovum will be best understood by regarding it as an amphibian ovum, in which the active pro-

toplasm has taken a peripheral position; and in so doing has avoided the necessity of splitting up a large mass of passive food material. *The central portion of the blastodisc represents the active portion of the pigmented hemisphere of the frog's ovum; while the marginal portion of the disc, together with the periblast, represents the active portion of the unpigmented hemisphere.* This correspondence is made evident by the course of events described in the sequel. The prevailing opinion, that the blastodisc alone undergoes cleavage, and that the periblastic cells have a different mode of origin, is entirely erroneous. The only difference between the holoblastic and the meroblastic types of cleavage, beyond what has just been mentioned, results from a difference in the rapidity with which the cleavage advances from the ectodermic towards the entodermic pole. In the frog's ovum the *first* cleavage-planes eventually reach the entodermic pole; in the teleostean ovum, the embryo begins to form before the cleavage reaches the equator of the ovum. But if the correspondence above stated be borne in mind, it will be seen that the equator of the frog's ovum does not correspond with the equator of the teleostean ovum, but with the marginal zone of the blastodisc. The ectodermic and entodermic hemispheres are not very unequal in the ovum of the frog; but in the teleostean ovum there is an enormous disparity between their morphological equivalents. The polar concentration of the active constituents of the ovum in the latter case has reduced the ectodermic hemisphere to a polar disc, and enlarged the entodermic hemisphere until it embraces nearly the whole sphere. As most of the material of the entodermic hemisphere lies in the margin of the blastodisc and the adjoining portion of the periblast, it is plain that it takes a direct share in the process of cleavage. Remembering then that *the virtual equator of the teleostean ovum lies in the marginal zone of the blastodisc,* we shall find it possible to arrive at clear and comprehensive views respecting the entire course of cleavage, the peculiarities in the origin and growth of the germ-layers, and the manner in which the embryo is formed.

In the mature ovum, the whole protoplasmic envelope of the vitellus is charged with minute shining granules, which render it partially opaque. A few minutes after fecundation nearly all the granules have dissolved, leaving the entire ovum completely transparent. The same remarkable change was observed in the ovum of the Blackfish.

At the time of clearing up, a shadowy ring, enclosing a clear central area, becomes visible at the ectodermic pole. Tilting the microscope to a horizontal position, a profile view is obtained which shows

that the shadowy ring is due to the polar aggregation of the protoplasm. The clear central space is the place in which the pronuclei will soon become visible. Our observations on the formation of the polar globules are not yet completed; and for the present may be passed over with the single remark, that these bodies do not escape through the micropyle in the case of Ctenolabrus.

At the time the pronuclei appear, the blastodisc has a low conical form, the rounded summit of which is directed towards the centre of the vitellus. The peripheral, basal portion scarcely swells beyond the niveau of the egg-sphere, and its margin thins out gradually into the periblast. There is a thin, but distinct perivitelline space separating the zona radiata from the blastodisc and the upper half of the periblast. The axis of the blastodisc is vertical, coinciding with that of the ovum. One or two polar globules are seen in the perivitelline space, at the octodermic pole. The fluid filling this space is not pure water, as shown by the fact that it becomes finely granular in acids, and stains in carmine solutions.

Pronuclei. — The earliest view of the pronuclei that we have obtained represents them as two equal spheres (.01 mm. in diameter) already in contact. The male pronucleus lies directly above the female, so that a line joining their centres would pass through the point of contact and coincide with the axis of the ovum. These bodies have a smooth and distinct outline, and a slightly granular composition. In general appearance they do not differ from the nuclei of the blastomeres.

In a mounted preparation of the blastodisc, which was killed thirty minutes after the ova and spermatozoa were mixed, the male pronucleus lies at some distance above the female pronucleus. The latter is spherical (.007 mm.) and surrounded by radial lines; while the former has an elongated ovate form (.009 × .004 mm.), the larger end of which is nearest the female pronucleus. This form of the male pronucleus recalls that of the "male aster," as described by Fol[47] in the ovum of *Sagitta Gegenbauri*.

We have several times watched the conjugation of the pronuclei continuously, from the moment of contact to that of complete coalescence. The two spheres flatten against each other, the line of junction remaining distinct until each has assumed the form of a hemisphere. This stage is reached in seven minutes after first contact. The line of junction now becomes obscure, first in the middle, then at

[47] Fol. Recherches sur la Fécondation, etc., 1879, Pl. X. figs. 6 and 7.

the ends; and at the end of eleven minutes, reckoning from the moment of contact, the coalescence is complete, and we have a perfect sphere .02 mm. in diameter. Within one minute this sphere undergoes a transformation, which ends in the formation of the first cleavage-amphiaster. *This amphiaster has a horizontal position, at right angles to the axis of the ovum.* From the moment when this transformation begins to the moment when the first cleavage-groove appears, is only eight minutes. Thus the conjugation of the pronuclei and the formation of the first cleavage-amphiaster require twenty minutes. We have found very little variation from these figures.

From the above measurements it is evident that the pronuclei increase somewhat in volume during the few minutes (10–12) consumed in the conjugation. A similar fact was observed by Bütschli in the ovum of *Nephelis* (No. 44, p. 6), and more recently by Mark in the ovum of *Limax* (No. 28, p. 220).

In the mounted preparation just referred to, I can discover no distinct astral lines around the body which we regard as the male pronucleus.*

It is an interesting fact, that *the first cleavage-spindle is parallel with the plane of junction of the pronuclei*, precisely as in the ova of many invertebrata. If this spindle were vertical, as supposed by Hoffmann, we should expect to see a rotation of the pronuclei, like that described by Auerbach in the ovum of Rhabditis. No such event occurs in the ova we are describing.

The Velocity of Cleavage. — From the appearance of the first cleavage-groove to the moment of hatching is but little more than fifty hours in any of the species we have followed. This period was only forty hours in *Ps. oblongus.*

We have traced the genesis of the amphiastral figures to the ninth generation in Ctenolabrus. The time that elapses between the first cleavage-amphiaster and the ninth generation of amphiasters was ascertained in one case to be two hours and sixteen minutes. The time between successive generations of amphiasters diminishes as the cleavage advances. Between the first and second generation twenty minutes passed; between the second and third, also twenty minutes; between the third and fourth, eighteen minutes; and from this point onward the time diminishes gradually, until, between the eighth and ninth generations, only fifteen minutes intervene.

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* Mark (l. c., p. 221) finds that the male pronucleus (Limax) is accompanied by an astral figure only in abnormal cases.

Van Beneden was the first to call attention to the fact, "that the time which elapses between two successive phases of cleavage is shorter and shorter as the cells diminish in volume, and in consequence of such diminution."* As only about twenty minutes elapse between the moment of first contact of the pronuclei and the appearance of the first meridian cleavage-groove, it is plain that we have no time for an equatorial cleavage to take place between these two events. Thus, at the very outset, we meet with facts that appear to be utterly irreconcilable with Hoffmann's account of the origin of the periblastic nuclei. It remains for us to show precisely how these nuclei arise; and to ascertain whether there are really two very different modes of cell-genesis introduced by the division of the first, or of any subsequent, cleavage-amphiaster.

The Growth of the Blastodisc at the Expense of the Periblast. — The moment the coalesced pronuclei enter the amphiastral phase of activity, an important change in the form of the blastodisc sets in, which gradually transforms it into a calotte-shaped mass in the course of the first four cleavage-stages. These form-changes are especially noteworthy, inasmuch as they confirm and emphasize the fact already stated, that *the first cleavage-amphiaster cuts the vertical axis of the blastodisc at right angles.* If this amphiaster had a vertical position, as asserted by Hoffmann, and the accompanying cleavage-plane a horizontal position, then the axis of the blastodisc would *lengthen* during the process of division. Now just the reverse of this takes place. The axis shortens so rapidly that within five minutes after the amphiaster appears (*Ps. melanogaster*) it is reduced to little more than half its original length. In order to watch the progress of these changes the microscope must have a horizontal position, so that a profile view may be obtained. The inner conical face of the blastodisc flattens very rapidly, and concomitantly the peripheral face becomes slowly more convex. At the end of five minutes (we are now speaking of *Ps. melanogaster*) the blastodisc has the form of a double-convex lens, with outer and inner surfaces of very nearly equal curvature. Two minutes later it has a meniscoidal form, the convex outer face almost or quite in contact with the egg-membrane, and the concave inner surface moulded to that of the vitelline sphere. It holds this form for about five minutes, during which it becomes somewhat thicker *at the expense of the periblast.* Ten minutes after the disappearance of

* Van Beneden first noticed this fact in the ovum of *Gammarus locusta*, and of the rabbit, No. 4, p. 46.

the first cleavage-nucleus, the first cleavage begins, not by a groove on the external surface of the blastodisc, but by one on the internal face. *The early cleavages are all introduced by grooves running from the inner towards the outer surface of the blastodisc.* These introductory grooves, which have hitherto been entirely overlooked, but which are very conspicuous in all the teleostean ova we have studied, reach their greatest height (which is seldom more than one third the thickness of the blastodisc) at the moment when the grooves from the external surface begin. We may call these *inferior*, in distinction from the external, or *superior* grooves. The peculiarity of these grooves is that they *precede* the appearance of the superior grooves, and that they recede and rapidly disappear after the superior grooves begin. The first of these inferior grooves begins as a shallow rounded furrow, but usually culminates in a sharp angle. At the moment of culmination we have several times been able to recognize a differentiated vertical plane extending completely through the blastodisc, from the edge of the inferior to the edge of the beginning superior groove. The plane of division is then already established before an actual separation begins. This foreshadowed plane of division is visible in mounted preparations quite as early as any traces of division in the chromatic elements of the nucleus.

In five or six minutes after the inferior groove appears, the corresponding superior groove begins; and as it deepens, the inferior groove closes up and becomes completely obliterated in the course of eight or ten minutes. By this time the superior groove has cut fully half-way through the blastodisc, and its walls begin to close together, so that the deeper portion of the groove, seen in profile, has the form of a vertical line, while its upper portion opens widely in a funnel-like form. It is at this time that the second generation of nuclei become visible. Gradually the linear portion of the groove lengthens, *upward* by the closure of the funnel-shape space, and *downward* by descending towards the inner surface. But the plane of division has not yet reached the floor of the blastodisc; nor does it do this until some minutes after two new amphiasters have formed, and the second inferior groove is already well advanced (twenty-one minutes after the first inferior groove). The floor is reached first of all at the point where the second inferior groove crosses the path of the first superior groove. Nearer the margin of the blastodisc, where this is continuous with the periblast, the floor is not reached until considerably later. During all this we have looked in vain for any distinct trace or shadowy indication of a lower stratum, which, according to Hoff-

mann, becomes split off by a horizontal plane of division before the first meridian cleavage appears. Equally fruitless has been our search for this Hoffmannian stratum in mounted preparations and sections. Not only is there no such splitting, but the whole course of events thus far is incompatible with such an operation.

A series of optical sections obtained by the aid of the camera, at intervals of one to four minutes during the first cleavage, is exceedingly instructive. At the time the meniscoidal form is reached, there is still no boundary line between the periblast and the blastodisc. The blastodiscal portion of the protoplasmic envelope thins out gradually, and passes with even outlines into the periblastic portion, which, after passing the equator, becomes very thin. At every step it is very evident that the blastodisc is thickening and broadening at the expense of the periblast. At the time the first cleavage begins, the blastodisc has an oval form, with only a broad, shadowy outline, as seen from the inner surface. As the cleavage-groove descends, the margin of the disc thickens and the adjoining periblast becomes thinner. At the time the two new nuclei appear, the outlines of the blastodisc begin to clear up; but they do not attain their sharpest definition until a few moments after the second amphiastral stage is reached. Seen from the surface, the blastodisc now appears to be separated from the periblast by a clear-cut boundary line; but a profile view shows that the continuity is still unbroken. The illusive appearance is due to the abruptness with which the thickened margin of the blastodisc rises above the periblast.

Soon after the second amphiastral phase appears, we notice that the blastodisc begins to expand in the direction of its shorter horizontal axis; i. e. in a direction at right angles to the coming (second) cleavage-plane. At the same time the margin of the blastodisc begins to lose its sharp outline in surface views, especially at those points in or near which the second cleavage-groove is to terminate. Optical as well as actual sections show that the blastodisc is thinning out at these points, so that the boundary line between it and the periblast is again only a little less obscure than at first. With the appearance of the third generation of nuclei, the outlines begin to clear up again, becoming sharper and sharper, until a maximum limit is reached soon after the introduction of the third amphiastral phase.

Thus towards the close of each of these earlier amphiastral divisions there is an obscuration of boundary lines, especially at the marginal extremities of the incipient cleavage-grooves, followed in each case by a gradual clearing up, which culminates in the early part of

the succeeding amphiastral phase. At every clearing up we observe that the blastodisc is increasing in size, while the periblast becomes thinner and thinner. There can be no doubt about the fact that the blastodisc actually draws the larger portion of the periblast into itself during the first four cleavage-stages.

We have seen that each cleavage-stage comprises two distinct acts: first, an *expansion* which accompanies the elongation and division of the amphiasters; and second, a *contraction* or *concentration* of the protoplasm around the new generation of nuclei. At each succeeding contraction, or *systole* as it might be called, a portion of the periblast becomes incorporated into the blastodisc; and the outcome of this is, that the latter thickens up and soon presents the form of a calotte. The question naturally arises, What determines these relations between the blastodisc and the periblast? It is evident that there is some attractive force in the blastodisc which is absent from the periblast; and further, that *this force, whatever it may be, increases during the cleavage.* Now the nucleo-plasm is the only substance, so far as we can ascertain, which is contained in the blastodisc and not contained in the periblast; and it is a very interesting fact that *this nucleo-plasm increases at a much more rapid rate than the blastodisc.* We cannot here enter into a discussion of the nature of the nuclei; but the conclusion is unavoidable that the attractive power of the blastodisc resides either (1.) in the nuclei; or (2.) in a *special* portion of the protoplasm intimately associated with the nuclei in the process of division; or (3.) in both. So far as the question we are here considering is concerned, it is a matter of indifference which hypothesis we adopt.

The cleavage process not only increases the number and volume of the nuclei, but it distributes them throughout the blastodisc. It is the establishment of these centres of attraction in the margin of the blastodisc, which accounts for the transformation of the blastodisc into a cap-like body. *Pari passu* with the multiplication of these centres, the blastodisc rises above the niveau of the egg-sphere. Its margin thickens up at the expense of the periblast, becomes steep at the end of the first cleavage, thicker and slightly rounded at the end of the second, more strongly rounded (forming a re-entrant angle with the periblast) at the conclusion of the third, and deeply constricted from the egg-sphere during the 16-cell stage. The progressive deepening of the re-entrant angle carries the zone of junction a short distance under the margin of the blastodisc, giving thus the appearance of a complete separation from the periblast. But the continuity is still preserved, and is destined to remain for some time to come.

The 16-cell stage marks an epoch in the history of teleostean development. The median plane of the embryo is given with the first cleavage-plane, so that a longitudinal and a transverse axis are determined from the outset; but a more precise orientation is not attainable before the 16-cell stage. At this time we are generally able (in Ctenolabrus) to recognize *antero-posterior* relations, and with these a *right* and a *left* side. From this stage onward we recognize also a *difference of constitution* between the *central* and the *marginal* cells.* This distinction is brought out clearly in most of our mounted preparations obtained by the use of osmic acid followed by the chrom-platinum solution. As the contrast becomes more pronounced as cleavage advances, and as it eventually ends in a decided histological differentiation, its appearance at this stage must be regarded as an early anticipation of events realized at a much later date.

The relations now established between the blastodisc and the periblast, although fundamentally the same, are not so simple and unmistakable as at first. A precise knowledge of these relations is not readily attainable by a study of the living ovum. Actual sections and mounted preparations are required for this purpose. Vertical sections of this stage show that only the twelve marginal cells rest on the yolk.

The shaded portion of Fig. 1 shows how much of the floor of these cells is in contact with the yolk. The four central cells and an adjoining zone of the marginal cells form the roof of a shallow cleavage-cavity, of which we shall have more to say farther on. The floor of this cavity is formed by a very thin stratum of the periblast. This sub-germinal stratum becomes apparent in some cases as early as the 8-cell stage. In some of our sections it is in contact with the central cells, but seldom shows traces of fusion with them. In Fig. 2 all these relations are accurately shown. The periblast (*p*) joins the marginal cells at their inferior outer angle, and from this point to the point (*cl*) which marks the limit of the cleavage-cavity there is not the slightest trace of a periblastic layer. This region may be designated as the *zone of junction* (*z*). No nuclei are to be found in any portion of the periblast at this time. As to the origin of the subgerminal periblast seen in this stage, we can state positively that it does not arise by a horizontal cleavage. It is a portion of the periblastic material which works its way under the blastodisc con-

* Rauber noticed this fact in the ovum of Gobius, but gave no explanation of it. Morph. Jahrb., VIII., p. 287.

comitantly with the formation and expansion of the cleavage-cavity. From this stage onward the history of the periblast is the history of the cleavage of the marginal cells. Our success in tracing this history is largely due to the methods employed. It was the differential staining before mentioned that first placed us on the right track.

One or two points insisted on by Hoffmann deserve notice in this connection. Hoffmann's ideas respecting the manner in which the cleavage of the teleostean ovum is introduced were fully anticipated by E. van Beneden, in 1878.* "Directly after fecundation," says Beneden, "the egg of the Osseous Fish divides into two very unequal cells, very dissimilar, differing in constitution and significance; the one is the germ which segments and from which the blastodisc is derived; the other is formed by the deutoplasmic globe, clothed, at least partially, by a thin layer of protoplasm forming 'the intermediate layer.'" (No. 4, p. 54.) Hoffmann brings, in addition to his observations, the following *a priori* consideration to the support of this view: —

"Wenn das Ei der Knochenfische eine Zelle ist, worüber man wohl nicht mehr streiten wird, dann ist es auch ganz natürlich, dass bei der eintretenden Furchung, bei der ersten Theilung in Archiblast und Parablast, der erste Furchungskern die Theilung einleitet, sonst würde hier der Fall vorliegen, dass eine Zelle sich theilte, ohne dass der Kern sich daran betheiligte und in dem einen Stück unverändert liegen blieb, während das andere Stück kernlos wurde." (No. 13, p. 126.)

These two citations are sufficient for the present to show precisely how this matter stood at the beginning of our investigations. The evidence produced by Hoffmann in favor of his and Van Beneden's view is of a very positive character, and can only be impeached by calling in question the accuracy of his observation. He claims to have seen the first cleavage-spindle in a vertical position, and that the first cleavage-plane takes place accordingly in a horizontal direction. Just after the division of the spindle, two nuclei were seen in the axis of the "Keim"; one lying in the floor near the vitellus, the other at some distance from this and directly above it. The subsequent division of the upper nucleus was accompanied by cleavage of the archiblast; but the division of the inferior nucleus was not attended by cleavage, and led simply to the formation of a multi-nucleated cell, the so-called parablast. The parablastic nuclei were seen in each of the subsequent stages of cleavage, and kept equal pace with the archi-

* Cf. Kupffer on Laichen und Entwicklungsgeschichte des Ostseeherings.

blastic nuclei in the process of division. Hoffmann admits that his observations are not complete, inasmuch as he was unable to follow the division of the first cleavage-spindle, and did not even find a spindle formation for the original parablastic nucleus. His failure in these particulars is attributable to his method of employing strong acetic acid (five to ten per cent), which renders the germinal disc opaque so rapidly that one can get only uncertain glimpses of what is going on in the interior. Mounted preparations, or actual sections of these early stages, would have given pictures of a much more reliable character, which would have served to confirm or correct impressions obtained by the acetic acid method. After reading carefully Hoffmann's account of the initiatory cleavage, we find that the following questions have been left either unanswered or in uncertainty: —

(1.) Does the supposed horizontal cleavage take at first the form of a circular groove?

(2.) What are the form-changes which accompany this cleavage?

(3.) Does the vertical axis of the blastodisc lengthen or shorten during the process?

(4.) Can the primary periblastic nucleus be demonstrated either by actual sections or mounted preparations?

(5.) Can any parallel for such a cleavage be found in any other class of animals?

With reference to the first question we are told, — "Das Ei von *Scorpaena* ist, im Vergleich zu den gewöhnlichen Zellen immer eine sehr grosse Zelle, und es wird also eine geraume Zeit dauern, bevor die Furche, welche alsbald Archiblast und Parablast von einander scheiden soll, so tief vorgedrungen ist, dass wirklich völlige Trennung beider Stücke folgt. *Bevor es hierzu kommt, hat sich der erste Kern des Archiblast, und wie mir höchst wahrscheinlich ist, auch der des Parablast, schon wieder in eine neue Spindel umgebildet.*"

Neither the figures nor the descriptions give us any very definite idea of the time or the manner in which this remarkable cleavage takes place. About the time the two primary nuclei (one of the archiblast, the other of the parablast) appear, we are informed that the blastodisc changes from a bi-convex to a plano-convex form; but whether this change accompanies the division of the first cleavage-spindle, or follows it, is left entirely to conjecture. Certainly there is nothing in such a change which would imply a lengthening of the vertical axis of the blastodisc; nor does it follow with certainty that the axis shortens. There is then, confessedly, a great deal of uncertainty in regard not only to details, but also to some of the more important

features of this supposed horizontal cleavage; and Hoffmann will probably agree with us that the *circumstantial* evidence ought to be more complete, before we concede such a fundamental difference in development between closely allied fishes.

If, however, it be claimed that Hoffmann's figures furnish conclusive evidence of his view, we shall have to admit that the first appearances favor this claim; but a somewhat closer examination of the text and the figures leaves a very different impression. A fair presentation of the question at issue compels us to call attention to Hoffmann's Plate IV. Figs 1–4, and the explanation of the same as given in the Tafel-Erklärung (p. 165) and in the text (p. 106). As it does not comport with the purpose and limits of this paper to give a complete historical sketch of his observations on the origin and development of the periblastic layer, we have selected for examination the plate which most fairly represents the grounds of his view.

Hoffmann states, in a very plain and direct manner, that Fig. 1 (Plate IV.) represents a 2-cell stage of his archiblast; we think this is what we have called the 4-cell stage, but cannot affirm this positively. It is also explicitly asserted that Fig. 2 represents a 4-cell stage (of the archiblast). Now this is assuredly an error. There are only two stages in the whole development which could give a view approximating that seen in this figure; namely, the 8-cell and the 16-cell stage. As this figure is only *about* five minutes later than Fig. 1, according to the explanation on page 165, it is quite impossible that Fig. 1 should represent anything earlier than our 4-cell stage. The next statement is certainly astounding; for it declares that Fig. 3 represents the 8-cell stage (we are speaking of the "archiblast"). We can assert with well-founded assurance that this figure cannot be said to represent any stage earlier than the 32-cell stage. The climax is reached in the twice-repeated statement (pp. 106, 165) that Fig. 4 represents a stage in which the "archiblast" is composed of 16 cells. Comment is unnecessary. Allowing that, as inadvertencies, these statements do not completely invalidate the figures, it may still be fairly claimed that they raise grave doubts as to the accuracy of Hoffmann's interpretation; and that they furnish us with a good reason for setting aside this interpretation, provided we can replace it with one that is more satisfactory.

We regard Fig. 1 as a 4-cell stage seen somewhat obliquely, so that two nuclei appear below the other two, as if they were in a subjacent stratum of protoplasm. The figure is illusory, and gives no idea of the relation of the blastodisc and the periblast at this stage.

Fig. 2 can be best explained as an 8-cell stage, in which one of the misplaced nuclei escaped notice. The inclination of the axis of the ovum and the method of treatment account for the illusive appearances and the failure to discover any continuity between blastodisc and periblast.

Fig. 3 is probably a 32-cell stage (it cannot be earlier). It represents four amphiasters belonging to marginal cells as if they were in a median plane.

Figs. 4, 5, and 6 combine surface views with optical sections. They become perfectly intelligible the moment we recognize the fact that the nuclei seen beneath the blastodisc occupy an entirely unnatural position. Their true position is superficial, near the margin of the blastodisc. These nuclei are undoubtedly true periblastic nuclei in Figs. 5 and 6.

Figs. 7, 8, and 9 of the same plate represent stages in the division of the *first* cleavage-spindle. Fig. 7 of this plate corresponds very nearly, if not exactly, to Fig. 3 of Plate III., the latter being another of those perspective figures with the perspective left out.

Fig. 10 is a puzzle: we know of no stage in the normal development of the teleostean ovum that would present *three* cells in an optical section.

According to Hoffmann's view, Fig. 7 ought to present two parallel amphiasters. His explanation of the omission of the hypothetical inferior amphiaster is, that, owing to its more central position, it could not be distinctly seen. Our view would account for its omission on entirely different grounds.

Hoffmann repeatedly calls attention to the fact that his "parablastic" nuclei keep an exactly even pace with the "archiblastic" nuclei in the process of division. This accords with our interpretation of Figs. 1 and 2; for in these early stages all the nuclei divide synchronously.

The Origin of Periblastic Cells. — With the 16-cell stage before described begins a most interesting chapter in the history of the periblast. Reserving a detailed description of the cleavage for the later and full account of our observations, we may here confine our attention to the more important events of this history. The virtual equator of the ovum, as we have said, lies in the marginal cells of the blastodisc, and may be supposed to coincide very nearly with the plane of division of these cells, as seen in Fig. 2. The peripheral half of these cells contains the larger part of the material which, at the outset, formed part of the periblast. Approximately speaking,

this half, together with the adjoining periblast, constitutes the entodermic hemisphere of the ovum. The difference of constitution between the marginal and the central cells, which is brought out by a differential staining, is manifest in sections, though not so decided as in later stages.

In passing to the 32-cell stage, the central portion of the blastodisc becomes two cells deep, each of the four cells *a*, *b*, *c*, *d* (Fig. 2) splitting horizontally, as shown by the vertical position of the amphiasters. The marginal cells divide obliquely, so that the outer half of each still remains in continuity with the periblast.

One hour after the 16-cell stage, we find the blastodisc three cells deep in the central portion; nearer the margin, two cells deep; and at the margin, one cell deep, as shown in Fig. 3.

At this time we find that the marginal cells, which are continuous at their outer and inner angles with the periblast, are still more strongly characterized than in the 16-cell stage. Their relations with the periblast are essentially the same. The central cells show the brownish tint characteristic of osmic acid staining, while the marginal cells are *much* lighter. In preparations stained with Grenacher's borax-carmine, the central cells take little or no carmine; but the marginal cells and the periblast stain well, thus bringing out a very decided contrast in color. This contrast in color extends to the nuclei, those of the central region being deeply browned, those of the marginal cells being stained red. The radial arrangement of the protoplasm around the nuclei is much more strongly accentuated in the marginal than in the central cells.

If the osmic acid is stronger, or is allowed to work longer than usual before transferring to the chrom-platinum solution, the contrast is often very much strengthened. In some of the mounted preparations, the central cells are very deep brown, almost black, while the marginal cells are light yellowish brown.

In most of the mounted preparations of this stage, the marginal cells appear to be well marked off from the periblast, although continuous with it; but we have one in which some of these cells have already entered the syncytial condition, which usually appears about two hours later. In this case they are considerably flatter than the marginal cell seen at the left in Fig. 3. But the syncytial condition is not yet fully established, coming and going as often as the cells divide.

Two hours after the 16-cell stage, the blastodisc is from two to four cells deep; and one or two marginal cells (in section) present the

characteristics before mentioned. At this time (Fig. 4) the surface cells, which are destined to form the epidermal layer, are more flattened than the deeper cells. It is not certain, however, that this layer is now *distinct* from the deeper cells. There are some very good grounds for thinking that some of the deeper cells eventually take a superficial position, assuming the flattened form.

Three hours after the 16-cell stage, we find a wreath of flattened cells encircling the blastodisc. Faint boundary lines are sometimes visible in the living ovum, but they soon disappear. Sections of the blastodisc at this time show that the wreath is composed of two concentric rows of cells, the inner of which lies beneath the margin of the cap, and hence is not easily seen in the living ovum. The inner row of cells is continuous with the thin periblastic floor of the cleavage-cavity; and the outer row is continuous with the larger external periblast. These cells (Fig. 5), derived from the marginal cells of preceding stages, are quite distinct from all others of this stage, if we except one or two usually seen above them; and, as they form the *Anlage* of the periblastic cell-layer, they may be called periblastic cells.

The one or more cells lying above the periblastic cells, which are also shaded in the figure, are less strongly individualized; but are, in many (not all) preparations, easily distinguished from the overlying cells. These cells, which we regard as the Anlage of the future entodermic layer, differ from the superjacent cells less than from the periblastic cells.

The periblastic cells multiply rapidly, the nuclei passing through the typical amphiastral phase at each division. As they increase in number, they spread in both directions, — inward, beneath the blastodisc, and outward, beyond the margin of the same, — thus forming the syncytial layer described by Kupffer, Van Beneden, Ryder, Hoffmann, and others.

When the entodermic ring begins to form (Fig. 6, *en*), we find that the periblastic nuclei have already spread far under the blastodisc. At this time there are no cell-boundaries around these nuclei, so far as can be ascertained from sections and mounted preparations. The periblast is now only a little thicker under the outer edge of the blastodisc than elsewhere; and no nuclei are found completely outside this thickened portion ("bourrelet périphérique," Van Bambeke).

The periblast then becomes a cellular layer as the result of one of the concluding acts of cleavage. The so-called "free nuclei"

neither arise *de novo*, nor from the division of the first cleavage-amphiaster, but in cells belonging to the margin of the blastodisc or blastoderm, which have at first well circumscribed boundaries. Some time before the embryonic ring appears, the inferior marginal cells flatten and form a wreath around the blastodisc. From this time onward these cells are entirely distinct from the blastoderm. Of their further history and significance something still remains to be said in the sequel.

It is hardly necessary here to call attention to the general importance of the discovery of the precise origin of this peculiar cell-layer, the history and meaning of which have so long been a standing puzzle, forming one of the greatest obstacles in the way of understanding the germ-layers of the vertebrates. We hope to be able to show later that this history is equally applicable to other meroblastic vertebrate ova.

The Order and Direction of Cleavage-planes. — The first four cleavage-acts, ending with the 16-cell stage, present some points of interest in addition to those already discussed under the head of "general remarks on the ovum." The direction of the first cleavage-plane is that of the axis of the ovum, whether this be vertical or not. When the ovum assumes its normal position of equilibrium, the axis is vertical, or nearly so, in the majority of cases; but this position is not universal, even among the teleostei. Gravitation of course influences the ovum as a whole, and may thus be said to control, *indirectly*, the directions of the cleavage-planes; but the idea that the first or any subsequent plane is vertical simply because this is the direction of gravitation, is in plain contradiction with the fact that cleavage-planes may form all possible angles with the vertical plane.

The *succession* of cleavage-planes, at least the earlier ones, in spite of all irregularities, presents a general uniformity or order. Is this order predetermined, *von vorn herein?* and how far is it allowable to speak of homologous cleavage-planes? The correspondence between the first cleavage-plane and the median plane of the embryo, which has already been ascertained in a considerable number of cases, favors the opinion that this cleavage-plane is homologous in at least all animals with bilateral symmetry. The evidence is, however, very far from being complete. It does not follow, because one cleavage-plane is homologous, that all the rest must be so. In comparing the teleostean and amphibian types of cleavage, we find no difficulty with the first two meridian planes of cleavage; but the homology of the third and of the fourth is not so obvious, while beyond this one would hardly venture to compare individual cleavage-planes. Rauber is the only

one who has undertaken a comprehensive comparison of these two types of cleavage;[48] and he was the first to show that variations in the cleavage of the frog's ovum occur which bring the two types together. While Balfour and others have supposed that the "equatorial" cleavage comes considerably later in the teleostean ovum than in the amplibian, and that therefore the *order* of the cleavage-planes is not the same in the two types, Rauber holds that they occur *only* in the latter, being replaced in the former by meridian cleavages ("Längsfurchen"). It is very rare, according to Rauber, that we have *true meridian* cleavages in the frog's ovum; the cleavage-planes usually called meridian do not seek the pole, but *shun* it. This is particularly true of the third and fourth meridian cleavages, the polar distance of which is often so great that they run nearly parallel to the first and second, presenting thus the teleostean pattern. According to this view, the third and fourth cleavage-planes of the teleostean ovum would correspond to the fourth and sixth of the frog's ovum, the two equatorial cleavages being skipped. The "Polflucht" theory of Rauber breaks the homology of the cleavage-planes between the second and third cleavage, and is in so far unsatisfactory. Balfour, in common with most authorities, has mistaken the first *concentric* cleavage, which occurs in passing from the 16-cell to the 32-cell stage, for the first equatorial cleavage.

We hold that the *order* of these cleavage-planes is identical in both types; and, accordingly, that the first, second, third, and fourth in the one correspond exactly to the first, second, third, and fourth in the other. "Polflucht" offers, to our thinking, no explanation of the origin of "parallel" cleavages in the ovum of the teleost. The real cause of an alternation from meridian to equatorial cleavage has never, so far as we know, been stated. The *equatorial* plane of cleavage is a *forced* one, and hence it *follows* the meridian cleavages. The meridian planes are the *natural* planes of cleavage; and the equatorial only a *dernier ressort*, introduced in accommodation to the elongated form of the blastomeres produced by meridian cleavage. The same is true of the concentric cleavages. The cell must elongate in order to divide; but it elongates in a vertical direction only when it is not free to do so horizontally. In the blastodisc of the fish ovum the necessity for a horizontal cleavage arises later than in the frog's ovum; and it naturally arises earlier in the central than in the marginal cells. The

[48] Rauber. "Neue Grundlegungen zur Kenntniss der Zelle." Morph. Jahrb., VIII., pp. 255–335, 1882.

so-called "parallel" cleavages (the third and the fourth) are as truly *meridian* as the first and the second, using this term with reference to the individual blastomeres, not with reference to the entire ovum. There can be but little doubt that each blastomere, whatever be its position, elongates during its division *at right angles to its axis* (assuming of course that each has poles of its own); and if this be so, the difference between *meridian* and *equatorial* is one of name only. In other words, a plane which may be called equatorial to the entire ovum may be truly meridian to the individual blastomeres. In regarding the "parallel" grooves as meridian, not to the ovum, but to the blastomeres in which they occur, we have, it seems to us, an explanation of these grooves that is in perfect accord with what is now known in regard to cell-division, and escape the necessity of appealing to an unknown factor, such as Rauber has had recourse to. His "polflucht" theory, ingenious as it is, is built upon a hypothetical tendency or force (polar repulsion?), the existence of which is, in our opinion, much more than doubtful.

Irregularities of Cleavage. — The first cleavage splits the blastodisc into two equal, or sub-equal blastomeres; the second cleavage may likewise be equal or sub-equal. The third cleavage, which usually runs parallel to the first, dividing the blastodisc into eight blastomeres arranged in two parallel and equal rows, sometimes results in an oval instead of the usual rectangular form, in which one cell is central and seven are marginal. During the entire season we found but one case of this kind. The next cleavage gave five central and eleven marginal cells. The cleavage was not followed further, but the ovum developed into a perfectly normal embryo.

Another very interesting variation in the 8-cell stage was met with only twice. The third-cleavage planes were not parallel to the first plane, but meridian (not only to the blastomeres but to the ovum), resulting in an oval figure with radial symmetry. The next cleavage was concentric to the pole of the ovum. One of these was mounted and the other was lost, so that we are unable to say how such a variation would end. The ova were supposed to belong to Ctenolabrus.

On July 7 an ovum belonging to Ctenolabrus was found a few minutes before the formation of the first cleavage-amphiaster, and watched as far as the 16-cell stage. The first three cleavages were quite regular, but the position of the eight blastomeres was sufficiently different from the normal, to induce a very unusual form of the 16-cell stage. This stage presented *seven* central cells and nine marginal ones. The ovum developed a perfect embryo.

July 27, ova and spermatozoa of the Blackfish were mixed. The ova proved to be immature, the cleavage halting at the 8-cell stage. The interesting feature of the first cleavage was, that the two blastomeres at the close of the division assumed a perfectly circular outline, and separated so far that a distinct space was left between them. During the second cleavage, the two blastomeres came in contact, but did not flatten against each other, as they ordinarily do. The third cleavage was very feeble, and was not fully completed.

During the sixth division, which results in the 64-cell stage, we have noticed an irregularity of some importance. This irregularity occurred in the division of the central cells. We found that in some cases one or more floor cells divided horizontally, the upper cell taking a position among the superficial cells; in other cases, the reverse took place, some of the upper cells dividing horizontally, and giving cells to the floor layer. From such facts we may infer that there is no histological difference at this time between the upper and lower layer.

All these irregularities might be interpreted to favor the ideas recently advanced by Pflüger; but we cannot admit that they confirm some of his more extreme conclusions.

Other Cleavage Phenomena. — The *cleavage-folds* ("corona plicarum" of Max Schultze) are generally seen to best advantage during the first and second cleavage; but occur during the third, fourth, and even the fifth cleavage as well. These folds are of the same nature and appearance as those seen in the early stages of the frog's ovum. They are the "Faltenkranz" of Reichert. They may be regarded as an outward expression of the radial phenomena which accompany cleavage, as has been suggested by one [49] of us, and as maintained by Van Bambeke.[50]

Three classes of *vacuoles* are seen in our mounted preparations. The first are thin lenticular spaces, bounded by the cleavage-faces of contiguous blastomeres. These are few in number, and reappear in successive stages, particularly the earlier ones. These vacuole-like spaces are present in the living ovum. They are filled with a fluid which does not stain. The second class of vacuoles are small, round or semicircular, and arranged along either side of the external cleavage-lines. They are not found on the inner surface, nor at points intermediate between this and the outer surface. Their

[49] C. O. Whitman. "Embryology of Clepsine." Quart. Journ. Mic. Sc., 1878, p. 41.

[50] Ch. van Bambeke. "Nouvelles Recherches sur l'Embryologie des Batraciens." Arch. de Biol., I., p. 366, 1880.

arrangement gives the cleavage-lines a somewhat moniliform appearance. They are not seen in cleavage-grooves until the division is completed. They are unstained. We have not seen these in the living ovum, but think they may have been overlooked, as the blastodisc is usually seen from the inner surface, from which the vacuoles would not be easily recognized. A third class of small spherical vacuoles may be seen at different depths throughout the blastodisc.

Balfour found in the elasmobranch ovum vacuoles similar to the second class.[51] The presence of these vacuoles, he says, gives the cleavage-furrows a "beaded" appearance. "Their appearance is that of vacuoles, and with these they are probably to be compared. There can be little question that in the living germinal disc they are filled with fluid. In some cases, they are collected in very large numbers in the region of a furrow. Such a case as this is shown in Plate I. Fig. 6 *b*. In numerous other cases they occur, roughly speaking, alternately on each side of a furrow. Some furrows, though not many, are entirely destitute of these structures. *The character of their distribution renders it impossible to overlook the fact, that these vacuole-like bodies have important relations with the formation of the segmentation furrows.*"

The "spaltartiger Räume" described by Oellacher[52] differ widely in appearance and position from this class, but may nevertheless be of a similar nature. They appear, however, more like the "differentiated plane" which precedes and marks the course of a cleavage-groove. Flemming[53] has described and figured vacuoles much more closely resembling those we have described.

About the time the second cleavage begins, we find *minute opaque granules* along each side of the first plane of cleavage. These are most distinct and most numerous towards the extremities of the plane. At the deeper part of the groove, just where this stops and is replaced by the precleavage line above mentioned, the granules are arranged in two linear and parallel rows, one on each side the dividing line. At a somewhat higher focus, their arrangement is much less regular. These granules are neither abnormal nor artificial. As to their nature and origin, our preparations give us no definite information; but they are probably equivalent to the "cell-plate" of Strasburger and the thickenings of the "interzonal filaments" described by Mark (l. c., p. 231).

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[51] Balfour. Development of Elasmobranch Fishes, p. 13.

[52] Oellacher. "Beiträge zur Entwicklungsgeschichte der Knochenfische." Zeitschr. f. Wiss. Zool., XXII., pp. 394, 395, 1872. (Pl. XXXIII. Fig. 22.)

[53] Flemming. Zellsubstanz, Kern und Zelltheilung.

Nuclei. — We have obtained a very nearly complete history of the nuclei as far as the 64-cell stage; but we have obtained this from mounted preparations of the blastodisc, which, on account of their thickness, do not admit of examination with very high powers. To this fact must be attributed our failure to analyze the various conditions assumed by the chromatic elements, with such detail and completeness as have been attained on more favorable objects by Flemming, Strasburger, and Van Beneden.

Contrary to what is seen in most of the figures of Flemming and Strasburger, the chromatine figures form only a minimal portion of the amphiasters. The first cleavage-nucleus is, in the living ovum, only .02 mm. in diameter. The amphiastral figure is always present before the outline of the nucleus is lost. The nucleus presents at first nearly a spherical form, then an oval form just before vanishing. Preparations of the nucleus in this oval or elliptical form usually show signs of division in the equatorial plane, which indicates that the chromatine fibres have already arranged themselves in two groups. At this time the precleavage plane is already established, and is coincident with the equatorial plane of the nucleus. About this time the nucleus becomes invisible in the living condition. The two nuclear plates move towards the opposite poles of the amphiaster, assuming a rounded contour just *before*, or at the moment of, reaching the edge of the polar areas. The polar areas, the centres of the asters, are irregular in outline, often amœboid in form, and stain very little or not at all. The spindle-fibres are very feeble, and scarcely distinguishable from the astral lines. The chromatic elements eventually reach the centres of the polar areas, but not until about the time these elongate to form a new generation of amphiasters. The astral lines are not lines in the strict sense of the word; for they appear to be made up of linear and somewhat fusiform elements, each having a radial direction, and thus producing the impression of radial lines. The spindle-fibres, in many cases, show a very feeble staining. The size of the achromatic polar areas forbids the idea that anything more than a small part of their substance is derived from the nucleus. Although the achromatic portion of the amphiaster appears to take the lead in the process of division, we are by no means certain that the chromatic elements do not play an *active* part. The extremely interesting investigations of Van Beneden[54] on the distribution of the chromatine in different

[54] E. van Beneden. "Recherches sur la Maturation de l'Œuf et la Fécondation." Arch. de Biol., IV., Parts 2 and 3, 1884.

phases of the nucleus, do not appear to support the opinion that the chromatine is merely passive food-material, as maintained by Brass.[55] The entire behavior of the chromatic elements during the process of division seems to be opposed to associating them with such passive material as food-yolk. The partial disappearance of chromatine in the nuclei of starved animals cannot be regarded as conclusive evidence that it is *surplus* nutritive material.

The early amphiastral divisions are very nearly synchronous; but it is rather rare to find the nuclei keeping equal pace sufficiently long to enable one to trace the exact genetic relationship of 64 blastomeres. We have obtained only a few preparations showing precisely 64 cells, and none containing exactly 128 cells. The periblastic nuclei, at the time when they first appear around the margin of the blastodisc, all divide nearly simultaneously, nearly all the amphiasters taking a radial direction.

The Cleavage-cavity. — Ryder has traced the history of the cleavage-cavity with much greater care and thoroughness than any other observer. He has not, however, given us the *early* history of this cavity. We have traced it from its beginning in the 4-cell stage up to the time when it becomes a spacious cavity, roofed by the expanding blastodisc and floored by the periblastic cell layer. During the cleavage stages it remains a very shallow cavity. Its outline, traced in Fig. 1, is quite distinct in most of our preparations and sections.

The Germ Layers.

The Periblast. — Among previous investigators, Rauber[48] has made the nearest approach to the discovery of the true origin of periblastic cells. His observations were made on hardened ova of Gobius, in which no periblast ("plasmodium") was found till towards the end of cleavage. At this time a differentiated ring of marginal cells ("Randschicht") was found, which were regarded, somewhat doubtfully, as the primary periblastic cells. The following remark will show how the case stood in his mind: —

"Die Centralzellen sind ihrerseits immer umsäumt von einer flachen Randschicht, als einem Rest der ursprünglichen Randschicht, von der sich neue Zellen abgeschnürt und den vier ersten Centralzellen bei gesellt haben. *Auf diese Weise kommt es bei Gobius, so viel ich aus*

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[55] Brass. Zool. Anz., VI., No. 156, p. 681, December, 1883; and Zeitschr. f. wiss. Mikroskopie, I., No. 1, pp. 39–51, 1884.

[48] Rauber, l. c., pp. 288–290.

meinem Material auf Grund von Schnittserien ersehen kann, zur Bildung jener Schicht, die als Plasmodium, Couche intermédiaire, sekundäres Entoderm, etc. bekannt ist. Eine vom Beginn der Furchung an als untere Keimschicht auftretende kernhaltige Protoplasmamasse, welche z. B. bei den Salmoniden so deutlich als Plasmodium des Keimes, oder sagen wir als primäres Entoderm auftritt, fehlt meinen Präparaten über Gobius."

Rauber appears to regard this mode of origin not only as doubtful, but as exceptional; for he straightway assents to the view maintained by Hoffmann, as true of teleostei in general, differing from him only in holding that the plasmodium is a part of the germinal disc, which from its destination, should be designated as "primary entoderm." The origin of the periblastic nuclei from marginal cells does not sustain Rauber's opinion in regard to the comparative rank of the plasmodium (l. c. pp. 300, 320).

Hoffmann maintains that the periblastic layer is separated from the blastodisc as the result of the first cleavage, and that it remains ever after distinct from it, taking no direct part in forming the embryo. In regard to its function, he comes to the conclusion, "in dieser Kernschicht die Werkstätte zu sehen, welche die Bestandtheile des Nahrungsdotters, des Parablast, assimiliret, um Zellen des Archiblast oder dem von ihm abstammenden Embryo in eine für die Ernährung geeignetere Form zu überreichen, mit anderen Worten, die an Kernen reiche Protoplasmaschicht des Parablast functionirt als *provisorisches Blut.*"* This view of its function is however not wholly original with Hoffmann; for a similar idea had previously found expression in the writings of several embryologists, among whom may be mentioned Balfour and Klein, and more recently, but independently of Hoffmann's observations, Ryder, Kingsley, and Conn. Hoffmann states that the blastodisc grows during cleavage at the expense of nutritive material brought to it by the periblast, thereby overlooking the fact that it grows at the expense of the periblast itself.

With reference to the origin of the periblastic nuclei, Ryder [56] has suggested that the periblast may retain some portion of "the original nuclear matter of the egg," which may be the source of free nuclei in the yolk. In a recent paper [57] he has stated more at length his views on the function of the periblast, which he calls the "*yolk hypoblast*,"

* l. c., pp. 136, 137.

[56] Ryder. Bull. U. S. Fish Com., I., p. 298, 1881.

[57] Ibid., II., pp. 183–187, 1882.

although contending that it forms no part of the real hypoblast. In former papers on the Spanish Mackerel and Silver Gar, he held to the opinion maintained by Kupffer and others, namely, that the periblast was truly hypoblastic: but finding no connection between it and the cells which form the alimentary tract, he concludes that it is only "a temporary and evanescent structure, which vanishes completely when the contained yolk material has been absorbed" (No. 57, p. 185), and that therefore it cannot properly be called "one of the primary embryonic layers."

In a recent paper (No. 21) Waldeyer has discussed at length the relations of the "archiblast" and the "parablast," claiming, in opposition to His, that they are one in origin. With reference to the origin of what we have called periblastic cells, he remarks (p. 31): "So far as my observations go, in thin sections of hardened preparations, nuclei, apparently free, are seen to appear in the pellicle of the teleostean ovum, as well as in the subgerminal yolk-layer, which multiply by division." The appearance of distinct cell limits around these nuclei at a comparatively late date, is defined as a "secondary cleavage." Speaking of the meroblastic ovum in a general way, he says: "The cleavage of the eggs of all those animals in which blood and connective tissue occur, is not uniform from beginning to end; but a *primary* and a *secondary* cleavage must be distinguished. The first divides the egg, so far as it is capable of cleavage, into a number of cells which are ripe for the formation of tissues. These form, then, the primary germ-layers. A remainder of unripe cleavage-cells (in holoblastic eggs), or of protoplasm which has not yet been transformed into cells (in meroblastic eggs), is left over." (pp. 47, 48.) A secondary cleavage later makes cells out of this remainder. "When the cleavage of the germinal disc is concluded, the pellicle [Rindenprotoplasma] and the subgerminal processes [Keimfortsätze] begin to break up into cells. These cells are smaller than those of the germinal disc, and naturally lie at first beneath the disc, especially beneath its margin, where they are imbedded in the white yolk, and also in the pellicle [Dotterrinde]. . . . . This process is very easily seen in the bony fishes, where it has often been described." (p. 15.)

With reference to the fate of the archiblast and the parablast, Waldeyer agrees, in the main, with His. He derives the blood and connective tissue from the "Rindenprotoplasma und aus den in den Dotter eingesenkten Protoplasmafortsätzen, den 'Keimfortsätzen,' wie ich sie genannt habe."

Kupffer, in his work on the Ostsechäring, also draws a sharp line

of distinction between "archiblast" and "parablast," based on the supposed "free origin" of the nuclei of the latter. He distinguishes two parts in the parablast, a "subgerminal plate" and a peripheral portion ("Rindenprotoplasma").

We have found nothing to justify the original parablast theory of His, nor can we accept the term "parablast" as defined by Kupffer, Klein, Hoffmann, or Waldeyer. The facts presented in this paper justify the opinion that the periblast represents a part of the entoderm. At the outset it is continuous with the blastodisc, into the margin of which it is progressively concentrated and thus brought under the direct action of cleavage. From the 16-cell stage onward it becomes more and more sharply differentiated, until at the conclusion of cleavage it takes the form of a wreath of flattened cells, destined to remain henceforth an independent layer. The nuclei of the cells multiply rapidly by so-called indirect division, and with each division the cells flatten, while their boundaries become less and less distinct. At length a thin nucleated "plasmodium," without any traces of cell limits around the nuclei, is formed. It is a veritable embryonic entoderm, the function of which begins and ends with the absorption of the yolk material. At least, we have thus far failed, as did Hoffmann and Ryder, to find any evidence that this layer shares in forming any portion of the permanent entoderm. From the time this layer becomes fully differentiated, it remains, at every stage, so perfectly distinct from every other portion of the embryo, that we see no ground for suspecting that it enters into any of the permanent embryonic layers.

The periblast is then a *true* yolk hypoblast, and is therefore, as Ryder hypothetically suggested, in all essential particulars, the homologue of the hypoblast of the yolk-sac of the chick. The chief difference between the bird and the teleost in this respect is, that in the former the periblast is continuous with the permanent entoderm, while in the latter its continuity is broken at a comparatively early date.

The Origin of the Entoderm. — On the question of the origin of the permanent entoderm of the teleost, the different views admit of being grouped into two great classes, according as they affirm or deny the participation of the periblast. Each of these classes may be subdivided into two: the first, according as the *whole* or a *part* of the entoderm is derived from the periblast; the second, according as the entoderm is said to arise by *delamination*, or by *invagination*, of the margin of the blastodisc.

The history of the origin of the entoderm, excluding the periblastic portion of it, is involved in that of the embryonic ring ("Randwulst," "Randzone," "Keimwulst," "Keimsaum"). We have given considerable attention to the formation and growth of this ring, with a view to obtaining an accurate idea of its composition and its relations to the embryo. Its mode of origin, composition, and history are the same in all the ova we have examined. Van Beneden, Hoffmann, and others, have insisted very strongly that this ring is not formed by a process of invagination. Balfour (No. 20, pp. 64–70) contends that there is no "true ingrowth or invagination of cells" in the elasmobranch development; and has devoted considerable space to an attempt to refute Haeckel's statements regarding such an invagination in teleostean development. On the other hand, Götte,[58] Haeckel (No. 3), Henneguy,[59] Kingsley, Conn, and Van Vleck, are very positive that the ring is formed by invagination, or ingrowth from the margin of the blastodisc; and we have satisfied ourselves that this view is essentially correct.

We do not affirm that any sharply delimited portion of the blastodisc is actually infolded; but we have *positive* proof that the ring arises as a centripetal *ingrowth* of cells from the margin of the disc. *Within one hour from the time the ring begins, the central area bounded by its inner edge is reduced to about one half its original extent, as shown by camera drawings of Ctenolabrus.* This fact furnishes indubitable evidence of the ingrowth, which has been so often denied and treated as incompatible with the facts of vertebrate embryology. A comparison of optical sections of the blastodisc, taken at short intervals during the formation of the ring, tells the same story. Actual sections simply furnish a verification of observations made on the living ovum. Precisely as was first shown by Götte, we find the ingrowing layer bending at the margin into the ectodermic layer. "Nach beendigter Furchung," says Götte, "bilden die Zellen des Keimes eine linsenförmige Scheibe, welche in einer entsprechenden Vertiefung des Dotters ruht. Darauf verdünnt sich die Mitte des Keimes und löst sich vom Dotter, so dass zwischen beiden die Keimhöhle entsteht, *Dann schlägt sich der Rand des Keimes auf einer Seite nach unten um und breitet sich an der unteren Fläche des Keimes aus. Dasselbe*

[58] Götte. Berlin. medicin. Centralbl., pp. 404–406, No. 26, 1869; and Archiv f. mikr. Anat., IX., p. 679, 1873.

[59] Henneguy. Bull. Soc. Phil. de Paris, 1880 (extract in Ann. Mag. Nat. Hist., IV., 1880). Compt. Rend., XCV., pp. 1297–1299, 1882 (abstract in Journ. Roy. Micr. Soc., III., p. 190, April, 1883).

geschieht später an der übrigen Peripherie. So besteht der Keim aus zwei Schichten, welche im verdickten Rande zusammenhängen." This description of the Trout corresponds exactly with Haeckel's account of the development of the Gadoid ovum: "Jetzt folgt der höchst wichtige und interessante Vorgang, den ich als *Einstülpung* der *Blastula* auffasse und der zur Bildung der Gastrula führt (Fig. 63, 64). *Es schlägt sich nämlich der verdickte Saum der Keimscheibe, der 'Randwulst' oder das Properistom, nach innen um und eine dünne Zellenschicht wächst als directe Fortsetzung desselben, wie ein immer enger werdendes Diaphragma, in die Keimhöhle hinein.* Diese Zellenschicht ist das entstehende Entoderm." (p. 439.) In one respect this account is incorrect; for it represents the entoderm as spreading beneath the entire blastodisc and forming a floor to the cleavage-cavity. There is a plain rolling-under, or involution, as an initiatory step in the formation of the ring; but we believe that the process is, in the main, more correctly described as an *ingrowth*, due both to a rapid multiplication of the cells, and also to the centrifugal expansion of the ectoderm. The floor cells of the cleavage-cavity are entirely periblastic, and have nothing whatever to do with the ring. The inner edge of the ring represents the limit of the ingrowing layer, which is thus confined to a narrow arc, precisely as in the elasmobranchs, according to Balfour's statements.

As to the significance of this inflected growth of marginal cells, there is every reason to believe that it is fundamentally the same phenomenon that has been so often described in other groups of animals as accompanying the epibolic or circumcrescent expansion of the ectoderm: in short, it must be regarded as the equivalent of a gastrula invagination. There is, of course, no such wholesale invagination as supposed by Haeckel, nor is such an invagination a necessary consequence of the view that the ring is the homologue of the lip of the blastopore in Amphioxus. Regarded as an invagination, the process is an extreme abbreviation of that seen in Amphioxus, since it is limited to an arc that agrees very nearly in width with the embryo (exclusive of the yolk-sac). If we take into consideration the embryonic entoderm (periblast) as well as the involuted entodermic ring, it would then be perfectly correct to say that there is a *complete* ingrowth; for the periblastic cells begin to multiply centripetally shortly before the ring appears, and reach the centre of the floor of the cleavage-cavity a little after it is formed. Thus the periblastic portion of the entoderm can be said to participate in, and to form a part of, the general ingrowth of the entoderm. That this ingrowth

takes place *before* the circumcrescent growth of the blastoderm is half completed, must be accounted for on the same general grounds that we should account for the formation of the embryo *before* the closure of the blastopore. From this standpoint, the fact that the periblast accompanies the blastoderm around the yolk becomes comprehensible and reconcilable with our general interpretation.

The investigations of Götte make it sufficiently clear that a similar ingrowth is characteristic of other meroblastic ova; and, contrary to the statement of Balfour (l. c., p. 69), we are confident that the development of the amphibian and the elasmobranch ovum furnishes nothing incompatible with this fact. The counter arguments drawn from this source will be considered in the memoir that is to follow this paper.

The epidermal layer of the ectoderm takes no share whatever in the involution. The entoderm bends directly into the deeper layer of the ectoderm, as is shown in Fig. 6, and as has been stated by Hoffmann and Henneguy. But this point can only be determined by sections; and this accounts for the error into which Kingsley and Conn have fallen, in supposing that the epidermal layer alone is inflected (l. c., p. 201).

An optical section, coinciding with the future median plane of the embryo, a few moments after the first indications of the ring appear, shows that the involution is not equally strong at the two opposite points of the ring. At the posterior margin, the in-rolling portion presents a strongly voluted outline; while at the anterior border it is much more feebly expressed. As the ring widens centripetally, we notice that the posterior thicker portion flattens and thins out as it spreads inward to form the "embryonic shield." The inward growth of the ring is completed in about an hour at all points, except at the posterior border, where the "shield" still continues its centripetal growth. Very soon after the ingrowth, which we may call the entodermic ring, to distinguish it from the ectodermic portion of the embryonic ring, has fairly begun, it appears everywhere to be only one cell thick, except in the axial region of the shield, where we find it from two to four cells deep. During the second hour of the ring, the shield, which represents the anterior end of the embryo (the hind end being represented prospectively by the remainder of the ring), becomes considerably thinner and nearly doubles its axial length. At the end of this time, it is only about three cells thick where it bends into the ectoderm; and from this point it becomes gradually thinner towards its anterior free edge, where it is only one cell thick.

Three or four hours after the appearance of the ring, when the blastoderm covers about one third of the yolk, and the anterior end of the embryo has nearly reached the ectodermic pole, we may obtain a good view of the formation of the chorda. For this purpose the ovum is so placed that we can get an optical transverse section of the embryonic portion near the middle. At this point the entoderm is only one cell deep, and the formation of the chorda can be seen with the greatest ease. A median band of the entoderm becomes plainly cut off from the lateral portions by vertical and nearly parallel planes of division. This median band, which is perfectly flat and of even thickness with the lateral portions, is the Anlage of the chorda. Leaving the history of the chorda at this point, we have to inquire how the alimentary tract is formed. As the lateral portions of the entodermic layer thicken up, they divide into two strata: the lower stratum is one cell deep, and represents the so-called "secondary entoderm"; the upper, which is several cells thick, represents the mesodermic plates. The lower strata abut at first against the inferior angles of the chorda, but close up under the chorda somewhat later, thus forming a single layer of flattened cells. About the time the blastopore closes, a median strip of this layer, corresponding in width to the chorda, begins to thicken, soon becoming two or three cells deep. It is this thickened strip that gives rise to the alimentary tube. Precisely how the solid band is converted into a tube, we are not at present able to state.

Comparing the foregoing account with that given by O. Hertwig [60] in his last paper on the development of the mesoderm of the amphibia, one important difference is seen. Hertwig comes to the conclusion that his "Chordaentoblast" forms not only the chorda, but also the roof, of the alimentary canal, the lateral and ventral portions of the canal being derived from the "Darmentoblast." We think that what we have described as the *entodermic ring* corresponds to the *chorda-entoblast* of Rana; and it seems plausible that the periblast should correspond to the "Darmentoblast" ("yolk hypoblast of Scott and Osborn [61]). On this view we should expect the periblast to take some share in forming the alimentary canal, which cannot be admitted if our observations are correct. Hertwig's observations do not bring the development of Triton and Rana into agreement on this point; and until this is done, we cannot expect to see the differences between the teleostean and amphibian development fully reconciled. But as there

[60] O. Hertwig. Jena. Zeitschr., XVI., pp. 247–323, 1883.

[61] Scott and Osborn. Quart. Journ. Micr. Sci., XIX., 1879.

seems to be a fundamental agreement in the development of all telolecithal vertebrate ova with respect to the origin of the "chordaentoblast," better called invaginate entoderm, or "invaginate hypoblast" (Scott and Osborn), there is a strong presumption in favor of the opinion that a complete agreement will yet be found to exist in regard to the precise origin of all the cells concerned in the formation of the alimentary tube. It is difficult to believe that the yolk cells ("Darmentoblast") form the *whole* of the mesenteron in Triton, only a *part* of it in Rana, and *none* of it in the teleost. Hertwig's observations on Rana have compelled him to admit that the invaginate entoderm takes a prominent share in forming the mesenteron; and his figures appear to us not only to warrant this conclusion, but also to suggest even more. Indeed, we think that they lend some support to the opinion that the mesenteron is formed exclusively at the expense of the invaginate entoderm.

Our observations on the relation of the mesoderm to the entodermic ring are not sufficiently complete to call for separate consideration. So far as they go, they appear to support the view that the mesoderm arises as two lateral masses, separated from each other by chorda cells, and from the periblast by a stratum of cells which, after uniting beneath the chorda in the manner before stated, are destined to form the mesenteron.

Kupffer's Vesicle. — Although we have been able to trace the entire history of Kupffer's vesicle in several species of ova, its significance remains as complete a puzzle as ever. Balfour homologizes this vesicle with the terminal dilatation of the "post-anal gut" of the Elasmobranchii, without, however, assigning any grounds for his view. The history of the vesicle is, in many respects, so entirely different from that of the "caudal vesicle" of the elasmobranch embryo, that we do not feel ready to accept this interpretation. The interpretations which have been offered by Kupffer and Henneguy are still more unsatisfactory, and need not be considered here.

Kingsley and Conn were the first to give an accurate account of the origin of this vesicle (l. c., p. 208); but they give us no information in regard to its subsequent history, and almost no details of its origin and growth. As they have stated, the vesicle arises by the fusion or confluence of a cluster of granules ("globules"). These granules are at first few in number (2–4), more or less angular, quite dark, and not more than .002 mm. in diameter. In general appearance, they are not distinguishable from the scattered granules seen in other parts of the ovum. In Ctenolabrus they appear soon after the embryonic ring

passes the equator, when the length of the embryo is about four fifths of the diameter of the ovum. They increase in number, grow larger, coalesce by degrees, and finally blend in a single bubble-like vesicle in the course of five hours. This vesicle, .01 mm. or more in diameter, more than doubles its diameter in the next hour and a half; and, steadily expanding, attains its maximum dimensions by the time the blastopore closes. It is somewhat variable in size and shape, but seldom more than .03 mm. in diameter, which is less than one tenth the diameter of the caudal vesicle of the elasmobranch. During all this time it lies beneath the chorda and the entodermic stratum, and has no sort of relation with any tubular structure whatever. As the alimentary canal is not yet in existence, it is difficult to see how this vesicle can be the homologue of a dilatation which arises *in*, and has no existence *outside of*, the post-anal gut. Ventrally and laterally it is bounded by periblastic material, but it has no cellular envelope in the strict sense of the word.

Soon after the closure of the blastopore, the vesicle begins to grow smaller, completely disappearing in the course of six hours. It is during this waning period that it steps into relation with the posterior end of the entodermic tract, which as yet has no lumen in any part. We have followed this portion of its history several times in different species of ova, and have satisfied ourselves that it is everywhere the same. At the beginning of the wane, about one third of its surface may be said to be enveloped by the entodermic stratum. At this time this portion of its surface (upper hemisphere) is considerably flattened, so that the entodermic envelope is really a very shallow umbrella-like concavity. Its vertical diameter now lengthens, while its horizontal diameter shortens; at the same time the entodermic concavity deepens, and its margin begins to form a plain constriction around the equatorial zone of the vesicle. Gradually the lower uncovered hemisphere rises up into the cavity, the whole vesicle growing rapidly smaller, until only a remnant remains, which is everywhere, except *perhaps* a small portion of its lower pole, enveloped by the entodermic layer. This remnant of the vesicle keeps on diminishing in volume, without any important change in shape, until it finally vanishes altogether. We are not able to say with certainty that a lumen exists from this time onward in this portion of the alimentary tract, but think this probable.

The closing history of the vesicle has thus some analogy with that of the caudal vesicle of the elasmobranch; but we require more evidence before concluding that they are homologous structures.

Secondary Caudal Vesicles. — Soon after Kupffer's vesicle begins to decline, we generally find a variable number of much smaller vesicles making their appearance between it and the hind end of the embryo. These secondary vesicles lie beneath the embryo in a somewhat thickened portion of the periblast. These grow larger, probably by coalescing, and may often be seen just in front of the terminal descending portion of the alimentary canal, until the tail attains a length of a millimeter or more. Whether the contents of these vesicles may be regarded as identical with the contents of Kupffer's vesicle, or whether any genetic relation exists between the two, we are not prepared to say. The general appearance of the two classes of vesicles is the same; and, until we had traced the disappearance of Kupffer's vesicle, we naturally assumed that the secondary vesicles arose by division of the primary vesicle, or that several primary vesicles coexisted from the outset.

Neurenteric Canal. — A surface view of the nearly closed blastopore shows that the epidermal cells surrounding it are much elongated in a radial direction. If the embryo be so placed that the remnant of the blastopore is seen obliquely, it will be seen to form a funnel-like depression, from the deeper and narrower portion of which a more or less distinct streak may be traced completely through this part of the embryo, which terminates at or near the posterior boundary of Kupffer's vesicle. In some cases this streak presents the form of a linear canal bounded by epithelium-like cells. The lumen of the canal is reduced to a mere line. It is difficult to recognize distinct boundaries to the wall of the canal in living embryos; and our sections have not thus far given us a satisfactory view of its relations to the alimentary tract. As the caudal plate (Ryder) thickens up, the canal, or streak representing it, appears to travel backward, which may be explained by supposing that the portion of the ring lying behind it is actually carried forward in order to form the hind end of the embryo. That such a migration of cells takes place is not absolutely certain; but the evidence is in favor of it, and the previous relation of the ring to the embryo sustains this view. There is at no time any nearer approach to a true neurenteric canal than we have described.

The Formation of the Embryo. — Our conclusions respecting the nature of the process by which the embryonic ring becomes converted into the embryo are essentially the same as those of His and Rauber. The so-called differentiation theory of Balfour and others fails to give any satisfactory account of the relation of the two lateral halves of the embryo to the ring, and offers no explanation of the "marginal

notch" which is sometimes seen in the blastoderm of the chick. The objections to the concrescence theory have been considered by one of us elsewhere: they are mainly drawn from those forms in which the evidences of concrescence have been partially obliterated, or more or less completely disguised. In the case of the teleostei, it is well known that the entire ring is converted into the embryo; but the manner in which this is accomplished has been very differently understood by different embryologists. The posterior end of the embryo is regarded by Oellacher as a fixed point throughout, from which the embryo lengthens *forward;* while His, on the contrary, holds that the anterior end of the embryo represents rather the "fixed point" from which the embryo lengthens *backward*, by the concrescence of the two lateral halves of the embryonic ring. It appears quite certain to us that the *principle* of concrescence underlies the formation of the embryo. The concrescence appears under the disguised form of a migratory movement of the cells, which accompanies the epibolic growth of the blastoderm. The direction of the movement of the cells composing the ring is that of concrescent growth. We have obtained two embryos showing a very well marked marginal notch at the posterior end of the embryo, in the place of the usually single caudal lobe. This notch, in one case, lasted for more than an hour, but was eventually obliterated. In the case of Elecate, Ryder has stated that the metameric segmentation extends beyond the embryo to the ring itself, which appears to give very conclusive evidence of concrescent growth.

The Relation of the Median Plane of the Embryo to the First Plane of Cleavage. — We have found it rather difficult, except in a few unusually favorable instances, to determine the relation of the first cleavage-plane to the median plane of the embryo; but we have very satisfactory grounds for the conclusion that the two planes coincide. Roux and Pflüger came to the same conclusion in the case of the frog; while Rauber was led to think that the two planes cut each other at right angles. This coincidence appears to hold true in the case of Rhabditis, according to Götte's figures. In a paper that has just come to hand, E. van Beneden [62] makes the following remark on this point: "Le fait que chez les Ascidiens et probablement aussi chez d'autres animaux à symétrie bilatérale, le plan médian du corps de l'animal futur se marque dès le début de la segmentation justifie pleinement

[62] E. van Beneden. "Recherches sur la Fécondation." Arch. de Biol., IV., fas. 3, p. 570.

l'hypothèse d'après laquelle les matériaux destinés à fournir à la moitié droite du corps siègeraient dans l'un des hémisphères latéraux de l'œuf, tandis que l'hémisphère ovulaire gauche engendrerait tous les organes de la moitié gauche du corps."

If our ideas of the promorphological relations of the ovum are well founded, it will be seen that we have a very satisfactory foundation for the opinion, first suggested by Balfour (Comp. Emb., II., p. 312), that the neural surface is identical throughout the metazoa. It is hardly necessary to add, that this view is in perfect accord with the theory of concrescence before mentioned. Indeed, it is difficult to see how one can hold to the former, and deny the latter.

EXPLANATION OF FIGURES.

Figs. 1–5, *Ctenolabrus.* **Fig. 6,** *Ps. oblongus.*

All magnified 280 diameters.

Fig. 1. Blastodisc seen from the inner surface. The Arabic numerals give the order of the cleavage-planes; the shading indicates that portion of the floor of the marginal cells which rests on the yolk. *a*, *b*, *c*, *d*, central cells; *cl*, boundary of the cleavage-cavity; *x y*, plane of the vertical section seen in Fig. 2.

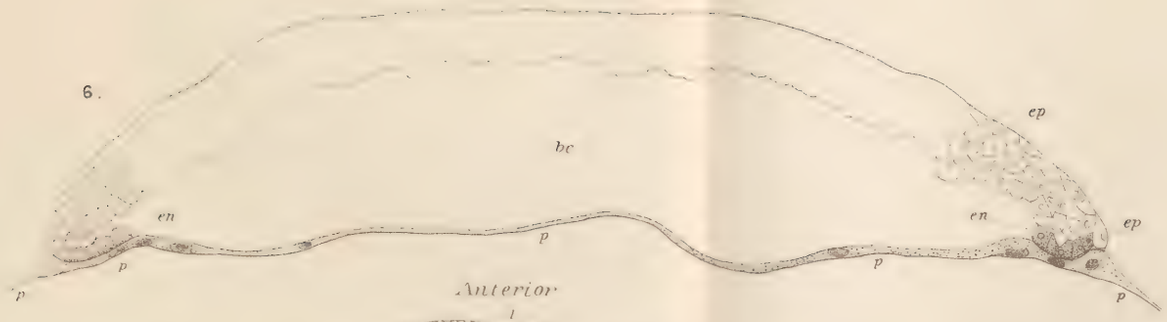
Fig. 2. Transverse section of the 16-cell stage, in the plane indicated by the dotted line (*x y*) in Fig. 1. *p*, periblast; *bc*, cleavage-cavity.

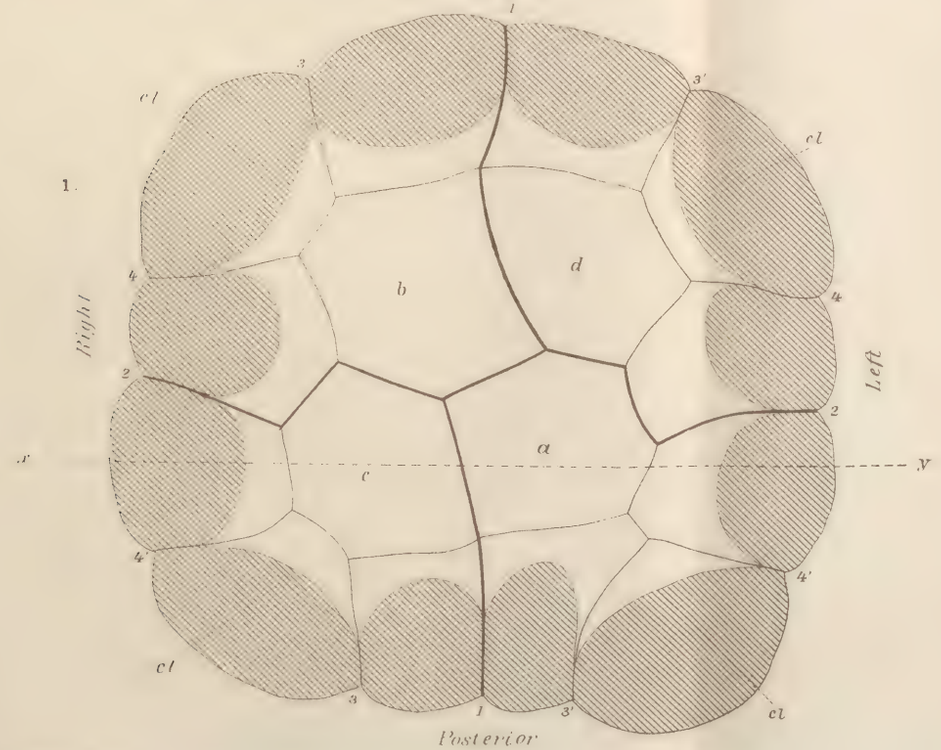
Fig. 3. Section of the blastodisc one hour after the 16-cell stage. The marginal cells are shaded.

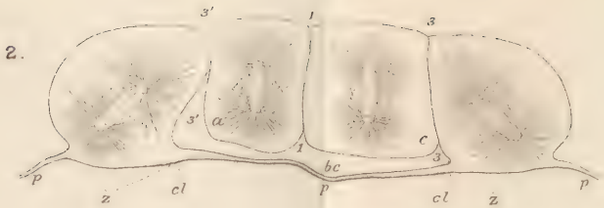
Fig. 4. Two hours after the 16-cell stage. *ep*, epidermal layer.

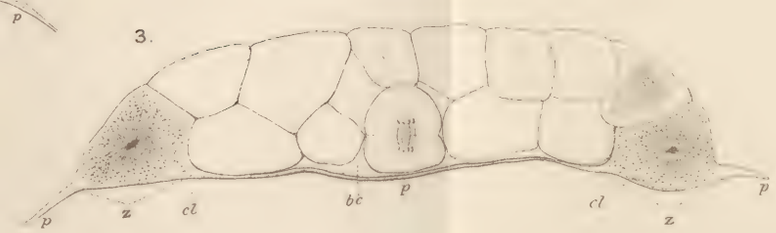
Fig. 5. Three hours after the 16-cell stage. The marginal cells assuming the form of periblastic cells.

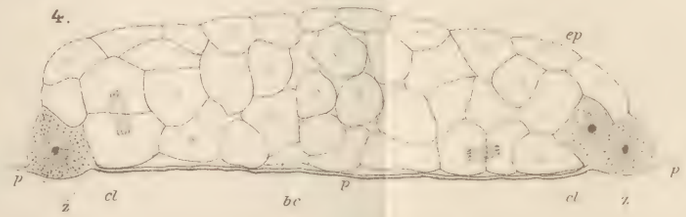
Fig. 6. Transverse section at the time when the entodermic ring appears. *en*, basis of the future entoderm.

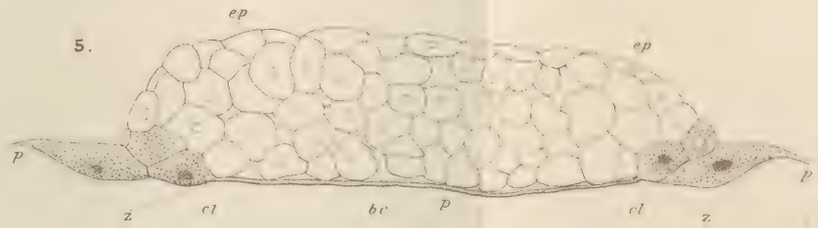












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